CLINICAL INVESTIGATION OF THE 1987-88 MASS MORTALITY OF BOTTLENOSE DOLPHINS ALONG THE U.S. CENTRAL AND SOUTH ATLANTIC COAST

final report to

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and
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and
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INTRODUCTION

From early June, 1987, until March, 1988, unprecedented numbers of bottlenose dolphins, Tursiops truncatus, washed ashore along the Atlantic coast from New Jersey to Florida. Details of the initial response to the event, subsequent organization of a multi-disciplinary team of investigators, and scope of the analyses were provided in an unpublished Interim Report submitted to the U.S. Marine Mammal Commission in May 1988. An account of the extent and impact of the mortality has been prepared by Scott et al. (1988).

The event was unparalleled, and therefore demanded a comprehensive investigation of proximate and contributing factors. Routine laboratory protocols were modified to meet rigorous research standards. Contributing laboratories with expertise in pathology, biochemistry, microbiology, virology, contaminants, and biotoxins performed analyses on coded samples from the dolphins. Specimens for contaminant and biotoxin analysis were mixed with controls from unrelated Tursiops and four other cetacean species. At the termination of each study, data were transferred to our laboratory at the University of Guelph, and integrated with identifying information.

This report describes how the investigative process evolved, and the evidence implicating a biological toxin as the proximate cause. The dolphins apparently were poisoned by brevetoxin, a neurotoxin produced by the dinoflagellate Ptychodiscus brevis, Florida's red tide organism. The dolphins were eventually infected with a host of bacterial and viral pathogens which produced an array of beguiling clinical signs.

MATERIALS AND METHODS

Specimen Collection

Over 740 bottlenose dolphins stranded along the Atlantic coast during the 11-month period beginning June, 1987 (Scott et al. 1988). Data or specimens from 347 of these were available for analysis by the investigating team. Studies on pathology, virology, microbiology, and chemical and biological toxicology were carried out only on freshly dead animals (Table 1).

To examine and obtain blood samples from live animals, four bottlenose dolphins were captured just offshore along Virginia Beach on August 16, and nineteen more between October 6 and 9, 1987. Blood samples were analyzed for hematology and serum chemical constituents including electrolytes, metabolites, enzymes, proteins and protein electrophoretic patterns, thyroid and adrenocortical hormones, and viral antibodies.

Pathology

Tissues for pathologic examination were fixed in 10% buffered formalin. Samples were processed through alcohol and xylene and embedded in paraffin blocks. Sections 5 μm thick were stained with hematoxylin and eosin, Masson's trichrome, Brown and Brenn, methenamine silver, Von Kossa, or periodic acid Schiff.

Selected samples of lung tissue were processed for electron microscopy. They were transferred to glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in acetone and embedded in epon. Thick sections, 0.5-1 μm, were cut on a Reichert-Jung Ultracut E ultramicrotome[1], and stained with methylene blue. Ultra-thin sections of subsamples were stained with uranyl acetate and lead citrate and examined on a Hitachi HS-9 electron microscope.

For energy dispersive x-ray analysis, samples of lung were processed without osmium tetroxide. Ultra-thin (90 nm) sections were collected on nickel grids, and examined in a low-background beryllium holder. Mineralized deposits were characterized for elemental composition using a Phillips EM 400T/STEM/TN (Tracor Northern) 5500 Series 1 Energy-dispersive X-ray Analyzer. Sections were bombarded with electrons for 100 live seconds at an accelerating voltage of 100 kV with an electron probe size of 400 nm. Beam current conditions were standardized for each analysis. Deposits were probed at three sites progressing from the core to the outer edge; an adjacent area of lung was analyzed for background elemental composition.

Virology

Specimens were submitted to the Eastern Virginia Medical School (EVMS), the USDA-NVSL at Ames, IA, and the National Institute of Health (NIH). At EVMS, under the direction of Dr. K. Somers, tissues and lesions from 12 dolphins were examined for the presence of viruses by electron microscopy, immunofluorescence, and cytopathic effects in tissue culture. Monoclonal antibodies specific for influenza A and B, parainfluenza 1 and 3, varicella-zoster virus, herpes simplex 1 and 2, and adenovirus were used to test for the presence of viral antigens. Tissue extracts were inoculated into cell cultures of monkey kidney, human skin, human carcinoma (Hep-2, A549), and mink lung.

At the USDA-NVLS, under the direction of Dr. L. Peterson and Mr. G. Gustafson, virus isolation was attempted on 54 tissue specimens from 29 dolphins. A 10 percent tissue suspension was prepared and inoculated into embryonating chicken eggs (ECE) and cell cultures (CC). The number of specimens inoculated into ECE was as follows: yolk sac route - 34; allantoic route - 27; chorioallantoic membrane route - 27. The number of specimens inoculated onto each cell line was: Vero-M - 49; McCoy -34; Madin Darby canine kidney - 9; baby hamster kidney - 10; bovine turbinate - 23; dolphin kidney - 3; dolphin skin - 2. The following is the number of specimens inoculated onto primary cell cultures: chick embryo

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[1]The use of brand names is not intended to indicate or imply an endorsement for the named equipment or product.

kidney - 17, rhesus monkey kidney - 9, swine kidney - 23, and swine buffy coat - 5. Each specimen was passed at least two times in cell culture and/or ECE. The ECE were observed for embryo death and the allatonic fluid was tested for hemagglutonating viruses, influenza and parainfluenza viruses. The cell cultures were observed for cytopathic effect and examined by electron microscopy for viral particles. Thirty-five of the original tissues submitted to the USDA-NVSL were also examined by electron microscopy for viral particles.

Responding to public concern that the dolphins might have been infected with retroviruses such as that responsible for AIDS in humans, Dr. R. Benveniste of the National Cancer Institute, NIH, examined 17 blood samples taken from live dolphins. Peripheral blood lymphocytes were co-cultivated with normal human peripheral blood lymphocytes, human lymphocyte lines HuT78 and MOLT3, human monolayer cell line A549, and canine monolayer cell line FCf2TH. These cell lines support the growth of almost all known mammalian retroviruses, including human immunosuppression virus. Table 2 summarizes the results of this and other efforts to isolate and identify viral agents in dolphin tissues.

Bacteriology

Bacteriological studies were carried out at USDA-NVSL, the Virginia Beach General Hospital, and the Center for Disease Control (CDC), Atlanta, GA. Tissues and swabs were submitted on wet ice. Swabs for aerobic culture were submitted in Cary-Blair transport medium (catalog no. 06-0452, Remel, Lenexa, KS). Swabs for anaerobic culture were submitted in anaerobic specimen collection kits (catalog no. 3650, Becton Dickinson, Rutherford, NJ). All specimens were processed as soon as possible after arrival at the laboratory.

Tissue specimens and swabs submitted for aerobic culture were inoculated onto marine agar (Difco, Detroit, MI), MacConkey agar (Difco), and heart infusion agar (Difco) supplemented with 5% defibrinated bovine blood. These media were incubated at 37°C for 24 hours and at room temperature for an additional 48 hours. Swabs for anaerobic culture were inoculated onto anaerobic blood agar (Dowell and Hawkins 1981), and incubated at 35°C in an anaerobic glove box (Forma Scientific, Marietta, OH). Plates were examined for anaerobes after 24 hours and 48 hours incubation. The freshest tissue specimens were also inoculated onto charcoal yeast extract agar (CYE, Remel) in an attempt to isolate fastidious organisms which might not grow on blood agar. The CYE plates were incubated at 37°C in a CO_2 incubator (Model 5200, National Appliance Co., Portland, OR) and examined daily for 1 week.

The methods used for the biochemical characterization of isolates were essentially those of Edwards and Ewing (1986) and Clark et al. (1984). For characterization of Vibrio isolates, the following media were supplemented with sodium chloride (3% final concentration): indole, methyl red, Voges-Proskauer, malonate, nitrate, gelatine, and decarboxylases (Moeller). Heart infusion broth containing 1% (wt/vol) carbohydrate, 3% (wt/vol) sodium chloride, and 1.0% (vol/vol) Andrade's indicator was used for fermentation tests. For salt

tolerance tests, nutrient broth containing increasing concentrations (0%, 3%, 6%, 8% 10%) of sodium chloride was used. All biochemical tests for Vibrio spp. were incubated at 25°C. Biochemical tests used for characterization of organisms other than Vibrio spp. were incubated at 37°C.

Serology

At the USDA-NVSL, serum samples from live-captured dolphins were tested for antibody as follows: bovine leukosis, equine infectious anemia, ovine progressive pneumonia and bluetongue by immunodiffusion (ID); contagious ecthyma, chlamydia and Coxiella burnetii by complement fixation (CF); equine rhinopneumonitis, equine coital exanthema and equine herpes-2 by serum neutralization (SN); vesicular stomatitis by CF and SN; and African swine fever by enzyme-linked immunoassay test.

At the OMAF-VLS laboratory, Dr. S. Carman conducted standard virus neutralization microtiter assays to determine titers for serum antibodies to canine distemper virus (CDV) in samples collected from 13 dolphins captured alive off Virginia Beach in October, 1987. Two-fold serial dilutions of heat-inactivated (30 min at 56°C) test sera were mixed with equal volumes of Onderstepoort strain of CDV virus (originally obtained from R.C. Povey, OVC), containing 100 $CCID_{50}$. The mixtures were incubated at 4°C for 1 h, after which Vero cells were added. Plates were incubated for 4-5 d at 37°C in a humidified CO_2 incubator. Sera were tested in duplicate, along with known positive and negative canine sera as controls. The titer was determined as the dilution of serum that completely inhibited virus replication in 50% of the wells, or the 50% end-point was extrapolated.

Toxicology - Chlorinated Hydrocarbons

Analyses were performed at the USDA-NVSL, in the laboratory of Dr. H. Nelson and F. Ross. To reduce contamination, specimens of liver, blubber, brain and kidney were removed as soon as possible after the carcass was opened. Tissues were wrapped in aluminum foil, placed in plastic bags, frozen at -20°C, and shipped to the laboratory where they were stored frozen for up to 1 year. Included for comparative purposes were specimens of stranded pilot whales, Globicephala melaena (4 mature F, 3 immature F, 1 mature M, 1 immature M, 2?), harbor porpoises, Phocoena phocoena (1 mF, 3 iF, 4?), humpback whales, Megaptera novaeangliae (2 mF, 3 iF, 1 mM, 1 iM, 1?) and three captive Tursiops (2F, 1M). The tissues were collected and stored as described above, except that they were placed into plastic bags, without aluminum foil, and storage times ranged from 2 to 10 years.

Five gram samples of blubber and melon (when available, cortex from melon was also taken) were shaved into thin (1 to 2 mm) slices, diced, placed into a tared 100 mL beaker and weighed. Liquid nitrogen (25 mL) was poured over the material to disrupt the cells. Once the liquid nitrogen evaporated and the beakers had returned to room temperature, 10 g of Na_2SO_4 was added as an abrasive to facilitate maceration and to scavenge moisture. A robust glass rod was used

to press and macerate the material against the bottom of the beaker. One hundred mL of methylene chloride ($MeCl_2$) was added, and the covered beaker gently agitated on a platform shaker (100 rpm) at room temperature for 24 hours.

The $MeCl_2$ was then filtered through filter paper into a tared evaporating flask. The beaker was rinsed twice, each time with 50 mL of $MeCl_2$, and the rinseates were filtered into the same flask, which was then placed in a rotary evaporator with a water bath temperature of 44 to 47°C to remove the $MeCl_2$. The residue was weighed to determine the amount of lipid.

Liver samples were homogenized in a Waring blender, and a 10 g sub-sample was combined with 20 g of Na_2SO_4. The slurry was mixed with a wooden stirrer, weighed, and dried at 80°C for 24 hours; moisture content was determined by reweighing the dried preparation. One hundred mL of $MeCl_2$ was then added, and after gentle agitation, filtered through paper into a tared evaporating flask. The beaker was rinsed twice with 50 mL $MeCl_2$ and the rinseates filtered into the same flask. The flasks were placed on a rotary evaporator to remove the $MeCl_2$, then weighed to determine the amount of lipid.

The lipid extract from each liver, blubber, and melon sample was dissolved in 10 mL of equal volumes of $MeCl_2$ and cyclohexane. For blubber and melon samples with lipid yield greater than 2 g, 1 g of lipid was weighed into a 15 mL glass tube and used for subsequent analyses. Five mL of the solution was loaded onto a gel permeation chromatograph (GPC) (AutoPrep^R, GPC Analytical Biochemistry Laboratory, Columbia, MO). The GPC was equipped with a 60 X 2.5 cm i.d. chromatographic column packed with a 60 g BioBeads (BioRad^R, Cambridge, MA) SX-3 resin in a 48 cm bed. MeCl2:cyclohexane (1:1) was pumped at 5 mL/min to elute the column. Samples were fractionated according to American Organization of Analytical Chemists (AOAC) Official Methods of Analyses (14 ed., 1984, Section 29.037-29.043). One hundred fifty mL of eluate containing chlorinated hydrocarbons was collected into an evaporating flask, and the solvent removed. Ten mL of petroleum ether (PE) was added in 3 aliquots to the residue and the solution transferred to a column containing 20 g Florisil^R (60/100 PR grade provided by U.S. Food and Drug Administration, Minneapolis, MN). Two fractions were collected (fraction A, 200 mL PE; fraction B, 200 mL 50/50/0.5, PE/MeCl2/acetonitrile, V/V/V) in flasks according to AOAC Methodology Section 29.015, and the solvent removed as described. The residue in each fraction was redissolved with three rinses totalling 8 mL of equal parts PE and acetone, and transferred to a 15 mL capped tube. The level was then adjusted to a final volume of 10 mL, after aldrin was added as the internal standard. Fractions A and B were then subjected to gas chromatographic analysis.

Polychlorinated biphenyls (PCB, as Aroclor^R 1260) were quantified from fraction A on a Perkin Elmer 8500 Gas Chromatograph equipped with a Ni63 electron capture detector, a Perkin Elmer As 8300 Autosampler (Norwalk, CT), and a 15 m x 0.25 mm DB-5 (J and W Scientific, Folsom, CA) fused silica capillary column with splitless injection. The carrier gas, 5% methane in argon, was delivered at a flow rate of 1.0 mL/minute. Separation was obtained using a temperature program from 150 to 240°C at 2°C/minute with a 5.0 minute post-injection hold at 150°C. The PCB's eluted in a time window from 21 to 52 minutes, and were identified by GC retention times using congener standards (Muir et al. 1988)

obtained from the National Research Council of Canada (Marine Analytical Standards Program, Halifax, N.S), and also with gas chromatography/mass spectrometry (GC/MS). Quantitation was done by summation of all PCB chromatographic peaks identified by comparison with an Aroclor® 1260 standard obtained from the U.S. Environmental Protection Agency (EPA) (Las Vegas, NV).

Chlorinated pesticides (DDT group and trans-nonachlor) were quantified on a Perkin Elmer Sigma 1 Gas Chromatograph equipped with a Ni63 electron capture detector, a Perkin Elmer As 300 Autosampler, and a 2 m x 2 mm i.d. glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA). The carrier gas was 5% methane in argon used at a flow rate of 40 mL/minute and the oven temperature was 200°C isothermal. Pesticides were quantified by comparing with EPA authentic standards. Trans-nonachlor and p,p'DDE were measured in fractions A and B, then totalled.

The identities of PCBs and pesticides were confirmed in selected liver, blubber, and melon samples on a Finnigan/MAT TSQ 70 Tandem Mass Spectrometer equipped with a 5 m x 0.25 mm DB-1 (J and W Scientific, Folsom, CA) capillary column with splitless injection operated at a flow rate of 1 mL/minute helium. Fragmentation was by electron impact or methane chemical ionization. Spectra were identified by comparing them to the NBS-NIH Mass Spectral Library and to standards. The lower limit of sensitivity was estimated to be 0.1 ppm for chlorinated hydrocarbons and 1.0 ppm for PCB. Values below these limits were considered zero in statistical computations.

Liver and blubber samples were processed in batches representing ten animals. A positive control was prepared for each batch from pesticide-free bovine fat with known amounts of chlorinated hydrocarbons added. Mean percent recovery and reproducibility for these spiked samples (X% ± standard deviation) was: p,p'DDE (liver) 90 ± 9; p,p'DDE (blubber) 85 ± 8; PCB (liver) 92 ± 7; PCB blubber) 87 ± 9; nonachlor (liver) 89 ± 8; nonachlor (blubber) 80 ± 7. No adjustments in results were made on the basis of these recoveries. True reproducibility measurements were obtained from analyses of five pairs each of blind duplicate liver and blubber samples, spaced throughout the course of the analyses. The average percent difference between the duplicate values was 14% for PCB, 12% for DDE, and 10% for t-nonachlor. The precision of the blubber lipid extraction was determined by conducting ten separate determinations on a randomly selected blubber sample. Average lipid yield was 67.5%, with a standard deviation of 1.4%.

Sub-samples of dolphin tissues or extracted lipid were sent to the laboratories of the Canadian Department of Fisheries and Oceans, Bedford Institute of Oceanography (BIO), Halifax, N.S. and Massachusetts Department of Public Health, Boston, MA for independent verification of DDE, PCB (total and congeners), and extractable lipid. Blubber lipid results from BIO were systematically lower for samples above 50%, and equivalent for those below that level. Liver lipid results were in excellent agreement, with an overall average percent difference of 15% for 10 samples. Reproducibility on 17 of 20 blubber samples for PCB and DDE was 15% and 13%, respectively, with three outlier results attributable to different blubber lipid yield. Liver PCB and DDE reproducibility was 20% and 13%, respectively, on 10 samples.

Toxicology - Elemental Analysis

Liver from the dolphins was collected and stored in the same manner as described for hydrocarbons. One gram of blended liver was weighed into a 15 mL teflon screw-top vial (AOAC Official Methods of Analysis, 14 ed. 1984, Section 49.A01 to 49.A05). Five mL of nitric acid (Mallinckrodt AR Select^{R}, Mallinckrodt, St. Louis, MO) was added, and the capped vial was positioned in a shallow glass dish containing 100 mL H_2O and placed into a 400 watt microwave oven, where the sample was digested for 2 to 3 minutes. After cooling, the material was filtered through filter paper into a 25 mL acid-rinsed volumetric flask; scandium was added as the internal standard.

Liver samples were processed in batches of ten. National Bureau of Standards reference materials (1577a and 1566) and normal bovine liver with lead, selenium, and mercury added were run with each batch. Quantification of the elements was carried out on a Perkin Elmer Model 6500 Inductively Coupled Argon Plasma Emission Spectrometer (ICP) equipped with a Czerny-Turner 408 mm focal length monochromator with holographic grating (UV, 2880 lines/mm and visible, 1440 lines/mm). Individual emission lines were as follows:

Element	Wavelength (nm)	Element	Wavelength (nm)
Copper	324.754	Cadmium	214.438
Zinc	213.856	Lead	220.353
Selenium	203.985	Mercury	194.227

Measurements were made using the sequential or graphic mode. Individual elements were quantified against primary standards prepared according to recommended procedures (Perkin-Elmer Procedure Manual) or obtained from a commercial source (Fisher Scientific Co., Pittsburgh, PA). Mercury standards were digested the same as the samples; all other standards were diluted from stock.

Toxicology - Biotoxins

Liver samples were taken from 18 of the freshest dolphins, selected to represent three arbitrary phases during the event: early (5 between August 8-26, 1987), middle (5 between September 18 - October 8, 1987), and late (8 from December 13 - February 19, 1988). These were tested in the laboratories of the Massachusetts Department of Public Health, for the presence of saxitoxin (STX), a water-soluble neurotoxin produced by a marine dinoflagellate which is responsible for paralytic shellfish poisoning (PSP). Three that died during capture in October, 1987, were tested as controls. Each test involves intraperitoneal inoculation of tissue extract into mice, as a bio-assay screening procedure. Samples found to be positive on bioassay are then processed by extraction and purification, and active compounds identified and quantified using high pressure liquid chromatography (HPLC).

Sixteen of the liver samples from the beached dolphins that were tested for saxitoxin, and one additional dolphin liver specimen, were also analyzed for brevetoxin. Controls included the three dolphins that died during capture, and 14 additional bottlenose dolphin samples - 6 that stranded along the mid-Atlantic coast between August and November, 1988; 3 from the Texas coast (one in Feb, 1987, two in March, 1988); 1 from Cape Cod, MA, (1983); and 4 captives (3 adults, 1 calf). Analyses were carried out in the laboratory of Dr. D. Baden, University of Miami. Samples sent to the laboratory were identified by code number, and their identity revealed to the laboratory only after test results were made available to the principal investigator.

At the time of the outbreak, the possibility of biotoxin poisoning was considered, and in August, 1987, we obtained bluefish, Pomatomus saltatrix, Atlantic croaker, Micropogon undulatus, spot, Leiostomus xanthurus, and red drum, Sciaenops ocellata, from the onshore weir fishery off Virginia Beach. These were tested for PbTx. We also analyzed menhaden, Brevoortia sp., and weakfish, Cynoscion regalis, taken from the stomach of a dolphin (KDL644) stranded south of Cape Canaveral on January 13, 1988, for PbTx. This was the only suitable sample of stomach contents available in the entire collection. After preliminary results showed the presence of PbTx in some dolphin samples, we obtained 2 silver seatrout, C. nothus, 3 Spanish mackerel, Scomberomorus maculatus, and 3 menhaden, Brevoortia smithii, caught off Vero Beach, FL, by the Florida Dept. of Natural Resources in late February, 1988. Viscera were analyzed from fish individually or as a pooled sample; selected specimens of flesh were also tested using the same protocol and criteria as for the dolphin liver samples.

Dolphin liver specimens (35-275 g) were received frozen. Each sample was homogenized, then dehydrated by steeping in 2 volumes of anhydrous acetone for 10 hours, followed by vacuum filtration on a Buchner filter using coarse-grade ashless filter paper. Dehydrated samples were homogenized twice in chloroform solvent, and the solvent was removed by filtration. The acetone and chloroform filtrates were combined, discarding the solid residue. Each filtrate was flash-evaporated, the residues were each resuspended in 20-25 mL 90% aqueous methanol and were solvent-partitioned twice with equal volumes of light petroleum. Methanol fractions were retained, adsorbed to 15-30 g dry silica gel, dried, and packed into flash chromatography columns over 100 g dry silica gel. The dry columns were eluted with 2 column volumes of anhydrous acetone, and the eluates were reduced in volume to 0.5-1 mL. Samples were rechromatographed as described above, using 50-100 mL chloroform:methanol:acetic acid (100:10:1).

All eluted solvent was flash-evaporated, each residue was applied to a 20 x 20 cm 1000 μm preparative silica gel thin layer chromatography plate, and plates were developed in acetone/light petroleum (30/70). One-cm-wide fractions (5%) of each developed thin-layer plate were scraped and bioassayed using mosquito fish, Gambusia affinis. Fractions which were lethal as determined after 48 hours were scraped, eluted with acetone, and rechromatographed on 10 x 20 cm 500 μm preparative silica gel thin-layer chromatography plates using ethyl acetate/light petroleum (70/30) as solvent. Fractions of developed plates were bioassayed as described above, and active fractions were eluted with acetone. Eluted fractions were dried under a stream of nitrogen, redissolved in 250 μL HPLC grade methanol, and were filtered using a 0.2 μm nylon filter. Samples were subjected to HPLC using a C-18 reverse phase column and 85% aqueous methanol as

mobile phase. Detection was by ultraviolet absorbance at 215 nm. Concentrations and identity of individual brevetoxins were determined by peak height, retention time, and comigration using brevetoxin standards prepared in the laboratory.

Results obtained using this protocol are generally adequate to confirm the presence of PbTx. Nevertheless, as an additional step, Fourier transform infrared transmission spectrometry was performed using a Mattson Instruments Cygnus 100 FTIR equipped with a Unix Starlab 2000 database and laser internal wave number standard. Extracts from one of the PbTx-contaminated livers were prepared in KBr pellets, and the spectra obtained were compared to those of authentic PbTx by computer-averaging of 32 sequential scans of each sample. Spectra were overlaid by computer and similarities were documented in the fingerprint region of each spectrum (1900-400 cm^{-1}).

RESULTS

Pathology

Necropsy and histologic findings in organ systems found to have the most consistent pathologic disorders are summarized on Tables 3 and 4. The study required numerous observers over a broad geographic area. Inconsistencies in reported findings were therefore inevitable. Despite the limitation, trends were noted in the condition of the stranded dolphins. Those that came ashore in August and early September 1987 had a range of skin lesions. Small blisters and pock-like craters were common over the head region, particularly around the lips and snout, and in the soft tissues of the mouth. The dorsal fin, flippers, and tail flukes were also affected to some extent. Rarely, the entire surface of the body was covered with round raised pox-like lesions measuring up to 1 cm in diameter. Histologically, the lesions consisted of vacuolation and swelling of epidermal cell cytoplasm with no involvement of the dermis. A viral infection was suspected, and though inclusion-like structures were occasionally noted, they contained no convincing evidence of virus particles. Results of viral isolation from representative lesions are reported below (see Virology).

A second type of skin lesion noted was the sloughing of large areas of skin, exposing underlying intensely reddened dermis. In some cases, large blisters formed and coalesced into broad sheets of epidermis floating on a fluid-filled bed. The epidermis could be peeled back as easily as a covering of cellophane. This condition could be distinguished, both by character and cause, from the pox-like lesions. These lesions were associated with thrombosis of dermal vessels, presumably caused by bacteria, fungi, or protozoa. This condition was one manifestation of systemic bacterial invasion which seems to have been the ultimate cause of death of many of the dolphins during the hot summer months. As time progressed, fewer of these lesions were noted, whereas the pox-like condition on the lips and snout was still evident in dolphins recovered in late February 1988.

Other findings in the dolphins were also related to septicemia, and particularly to the effects on blood vessels which had been injured by bacteria. The vessel walls became fragile and necrotic, and were unable to contain blood. Plasma leaked into tissue spaces causing edema in many of the organs, and accumulation of massive quantities of blood-tinged fluid in the thoracic and abdominal cavities. Affected organs underwent necrosis as a combined effect of impaired circulation and bacterial toxins. In some cases the animals appeared to have died during the acute phase of bacterial infection. Others, less severely affected, had a more protracted illness which terminated in pneumonia, cerebral hemorrhage, secondary invasion by fungal organisms, and vascular collapse or shock.

Chronic lesions were present in some of the first animals examined in early August 1987. These were typically found in the lung, liver, pancreas, and heart, and were characterized by fibrosis. Specifically there was pulmonary and pleural fibrosis, hepatic capsular and parenchymal fibrosis, and myocardial scarring, most common in the subendocardial region. Pancreatic fibrosis grossly typical of chronic parasitic infestation was also present. Fibrosis in the lung was most severe sub-pleurally and much of the "pleural" thickening was actually due to this lesion. In the few animals in which the trachea was examined histologically, chronic tracheitis was consistently present.

Another remarkable and almost constant lesion was the loss of epithelium from pulmonary bronchioles. The walls of affected airways were lined by fibrous tissue in which mineralized debris was embedded, while the few remaining epithelial cells were stretched to cover the ulcerated surface. The mineralized structures, which measured 22-26 μm in diameter, were formed of concentric rings, with mineralization most apparent in the core. Electron-dispersive analysis revealed that calcium and phosphorus were the principal elements in these structures. Their concentrations decreased progressively towards the edges of the structures, and were undetectable in adjacent lung tissue.

In liver there was thickening of the capsule and fibrosis of parenchyma especially around portal triads and under the capsule. Some of this fibrosis was associated with parasitic infestation but elsewhere the fibrosis was typical of post-necrotic scarring. In several animals dying late in the outbreak there was severe hepatic lipidosis, hepatocellular anisokaryosis and single-cell necrosis consistent with toxic hepatopathy.

In many dolphins lymphoid follicles in spleen, lymph nodes, and intestine were depleted. The centers of the follicles were hyalinized, and lacked lymphocytes.

Bacteriology

A wide variety of bacteria was recovered from stranded dolphins (Table 5). The organisms include members of the genera Edwardsiella, Streptococcus, Vibrio, Pseudomonas, Klebsiella, Acinetobacter, Bacillus, Staphylococcus, and others. There was no particular pattern to their distribution within an animal. Members of the Vibrio group predominated, representing 52% of the total isolates. All tests for Chlamydia were negative.

Virology

Tissue specimens and lesions from dolphins were evaluated for the presence of viruses by electron microscopy, immunofluorescence, and cytopathic effects in tissue culture. No virus particles were observed in direct examination by electron microscopy, nor were antigens detected to influenza A and B, para-influenza 1 and 3, varicella-zoster virus, herpes simplex 1 and 2, and adeno-virus. All samples were negative for bovine leukosis, bluetongue, contagious ecthyma, equine infectious anemia, equine rhinopneumonitis, vesicular stomatitis, and ovine progressive pneumonia. There was no evidence of retrovirus infection.

Papovavirus was detected in 4 of 12 dolphins, on the basis of electron microscopic examination and cytopathic effects (CPE) in primary monkey kidney cell cultures inoculated with tissue extracts. The same extracts had no effect on human skin cell cultures, human carcinoma cell lines, or mink lung cell cultures. The virus was immunologically related to simian virus 40 (SV-40) as demonstrated by immunofluorescence with antiserum specific for the VP 1 capsid antigen of SV-40. Uninfected monkey kidney cells were negative for virus particles by EM and SV-40 capsid antigens by immunofluorescence. Herpes-like particles were also isolated from a mouth lesion from one of these dolphins. At the EVMS, Dr. Somers isolated a virus related to the reovirus family, from the palate ulcer of dolphin WAM-253. The virus has a restricted host range and induces cytopathic effects in dolphin kidney cell cultures (CCL 78) (American Type Culture Collection, Rockville, MD), but fails to cause cytopathic effects in human fibroblast or epithelial cells, mink lung cells, monkey kidney cells, and rabbit kidney cells; uninfected dolphin cell cultures show no evidence of the virus. The CPE occurred after a 2-3 day latent period, were reproducible, and consisted of cell clumping, apparent cell-to-cell fusion, ballooning degeneration, and lysis. Ballooning cells extruded transparent cytoplasmic extensions from the surface membrane. Electron microscopy of infected dolphin cells (CCL 79) (American Type Culture Collection, Rockville, MD) revealed the presence of virus particles 75-80 nm in diameter which accumulated in the cytoplasm. There were no intranuclear forms. The size, shape and localization of the virus was consistent with a reovirus identity. Reovirus-like particles were isolated from a palate ulcer from a fifth dolphin. The isolate induced reproducible CPE in dolphin kidney cell cultures. All three isolates are being further characterized.

Serology

Canine distemper virus-neutralization assays on serum from live-captured dolphins showed inhibition of virus in 6 of 13 samples. Titers greater than 1:2 suggest that CDV antibody was present. One dolphin had a titer of 1:128, two of 1:24, two of 1:12, and one of 1:6. There was no apparent bias in sex or age of the positive dolphins.

Biotoxins

There was no evidence of saxitoxin on preliminary mouse bioassay of dolphin liver samples. No further analyses were performed.

The results of brevetoxin analyses are presented on Table 6. The analysis consists of three purification steps, each followed by a fish bioassay. A negative result at any stage terminated the test. Only those samples positive in the third bioassay were subjected to HPLC. Diagnosis was based on detecting a specific HPLC peak which co-migrated with the brevetoxin standard. Fourier transform infra-red transmission spectrometry performed on the extract from dolphin WAM-280 provided unequivocal evidence that the active component was PbTx-2. Comparison of the generated wave numbers revealed characteristic absorption in the fingerprint region for both samples at 3435-3441, 2940-2941, 2851-2874, 1638, and 1056 cm^{-1}.

Using these criteria, eight of 17 stranded dolphins collected during the event tested positive for the neurotoxin; two of six collected near Virginia Beach in July and August, 1987; three of five in the same area between September 18 and October 8, 1987; and three of six along northern Florida in January and February, 1988 (Table 6). There was no apparent correlation between the concentration of the toxin and the chronology or location of stranding. No PbTx-2 was demonstrated in any other dolphin liver sample, including the three animals that died during capture in October, 1987.

Brevetoxin was found in the viscera but not in the flesh of menhaden taken from the stomach of dolphin KDL644; no toxin was detected in weakfish also taken from the same animal, nor from the liver of that dolphin. Of the seven species of fresh-caught fish tested, only the viscera of menhaden landed on February 20 and 28, 1988, contained detectable brevetoxin, at levels representing 200 μg per fish.

Toxicology - Organochlorines

Results of organochlorine analyses and lipid recovery in blubber and liver are expressed on a lipid weight basis, and are shown on Tables 7 and 8. The findings for liver are also expressed on the basis of wet weight (Table 9). Three major groups of organochlorine contaminants were detected: DDTs, chlordanes and PCBs. The DDT fractions included p,p'DDE, o,p'DDE, p,p'DDD, o,p'DDD, p,p'DDT, and o,p'DDT. This group is represented by p,p'DDE. Chlordane components included trans-nonachlor (t-nonachlor), cis-nonachlor, cis-chlordane, trans-chlordane, heptachlor epoxide, oxychlordane, and heptachlor. T-nonachlor was the major component and was selected to represent this group. The chromatographic profile of the PCBs was most like that of Aroclor ^{R} 1260, and consequently is expressed as such. In the following discussion, liver and blubber concentrations are expressed on the basis of lipid weight unless otherwise stated.

The majority of liver samples contained less than 10% extractable lipid (Fig. 1). The few samples that exceeded that value ranged up to 41%; all animals with greater than 15% extractable lipid were immature. The majority of blubber

samples contained more than 50% extractable lipid (Fig. 1); values averaged 10% higher in immature males and females than in mature animals (Table 7). Data from the three dolphins with extractable blubber lipid less than 10% were atypical, and were excluded from the calculations of mean values.

Average concentrations of organochlorines in blubber were higher in immature than in mature females, and showed the opposite pattern in males. Statistically (Newman-Keuls ANOVA), the difference between mature males and females was significant for DDE and PCB ($p < 0.01$), and t-nonachlor ($p < 0.05$); other statistical comparisons are shown on Table 10. The highest concentration of residues was in a mature male with 1.3% lipid in blubber; PCB was 6800 ppm, DDE 2000 ppm, and t-nonachlor 400 ppm. There was a significant correlation (linear regression $p < 0.001$) of PCB with DDE, PCB with t-nonachlor, and DDE with t-nonachlor in blubber of all animals. The blubber of captive dolphins had PCB levels comparable to those of the immature animals; DDE and t-nonachlor levels were comparable to those in mature males. The blubber lipid of the other cetacean species had significantly lower PCB than the stranded dolphins; there was no consistent pattern for the other contaminants.

The concentrations of contaminants in liver lipids of T. truncatus (Table 8) had a pattern similar to that in blubber. Levels of DDE were lower in mature females than in immature ($p < 0.05$) and mature males ($p < 0.01$). The captive dolphins had higher DDE levels than the average for the stranded group as a whole. Mature females also had lower values for t-nonachlor than immature males ($p < 0.05$). The male with the highest levels of organochlorines in blubber also had the highest concentrations in liver - 5200, 1300, and 200 ppm for PCB, DDE, and t-nonachlor, respectively. These data were omitted from statistical computations so as not to skew the population mean. In the stranded pilot whales, PCB concentrations were below detectable limits, and DDE and t-nonachlor were significantly lower than in all but the mature female dolphins. As in blubber there were significant correlations ($p < 0.001$) among all three classes of compounds in all groups of animals.

In the stranded dolphins, concentration of residues in liver lipid did not correlate with the amount of extractable lipid from that organ (Fig. 2). However, none of the dolphins with liver lipid concentrations greater than 15% had PCB concentrations above 200 ppm, whereas those with less than 15% liver lipid had up to 750 ppm.

Liver and blubber residues in individuals were compared to assess the capacity of the liver to process the compounds. Three patterns were evident. 1) For PCBs, a number of dolphins had higher concentrations in liver than in blubber, indicating that liver was not eliminating compounds at the same rate at which they were being delivered from the blubber (Fig. 3). 2) DDE residues in some animals were higher in liver than in blubber, perhaps for the same reason, but also because liver metabolizes DDTs to DDE, and therefore contributes to the DDE load at that site (Fig. 3). 3) Only two individuals had higher t-nonachlor in liver than in blubber, suggesting that liver can process it as it is delivered. In fact, the compound was undetected in many liver samples, perhaps indicating its rapid metabolism or excretion.

Liver and blubber from 11 dolphins were analyzed for individual PCB congeners. The representative distribution of each congener was similar in both tissues, and consistent with findings from other studies (Muir et al. 1988). Generally, congeners 138, 153 and 201 were the most highly concentrated. Excluding from the sample one dolphin with the lowest extractable blubber lipid, liver concentrations were 1.6 to 38 ppm, 2.9 to 43 ppm, and 0.3 to 27 ppm for congeners 138, 153, and 201, respectively; those in blubber were 2 to 75 ppm, 2.7 to 100 ppm, and 1.3 to 18 ppm, respectively. Within individual dolphins, the ratio of 138/153 was consistently and significantly ($p < 0.001$) higher in liver than in blubber. This pattern might be attributed either to more rapid mobilization of 138 from the blubber, or reduced ability to clear it from the liver.

Brain samples from 18 stranded animals were analyzed for organochlorine residues using the described technique. Concentrations (wet weight) of PCB, DDE, and t-nonachlor were 0-4 ppm, 0-0.4 ppm, and 0-0.3 ppm, respectively, and did not correlate with levels in other tissues. Values were consistent with or lower than reported for other marine mammals (O'Shea et al. 1980).

Toxicology - Heavy Metals

Liver concentrations of heavy metals are presented on Table 11. No significant differences were noted in comparisons among immature and mature, and male and female dolphins. As in other species, mercury and selenium levels were highly correlated (Muir et al. 1988); all values for heavy metals were comparable to those reported for other odontocetes (Honda et al. 1983, Muir et al. 1988).

DISCUSSION

This has been the most extraordinary saga of cetacean disease on record. Between the time the first dolphin stranded in New Jersey in June 1987, and the last on Florida's east coast eleven months later, over 740 animals died. The exact toll is not known, since almost certainly some animals were not recovered. However, Scott et al. (1988) estimated that 50% or more of the coastal migratory stock between Florida and New Jersey died during this period. Without a guiding precedent to help uncover the cause, it was necessary for the investigation to sweep a broad range of disciplines before settling on the eventual path to the probable solution. The two most likely potential causes for an outbreak of this kind were considered to be infectious disease and poisoning. After weighing evidence from 18 months of field and laboratory analyses, we have concluded that brevetoxin, the neurotoxin produced by the dinoflagellate Ptychodiscus brevis, probably was the proximate cause of this devastating event.

Early findings led the investigators away from microbial agents as the principal cause of death. There was no single pattern of illness that could be associated with a known pathogen, though it was clear that infectious agents contributed to and sometimes dominated the clinical picture. The first animals to come ashore on Virginia Beach in late summer clearly had been ill for some time, with a condition that ultimately affected skin, liver, and lung, and led

to the accumulation of fluid in the abdominal and thoracic cavities. Meanwhile, in New Jersey, Drs. W. Medway (University of Pennsylvania) and D. Roscoe (New Jersey Division of Fish, Game and Wildlife) indicated in personal communication that carcasses there were in better condition and less affected with secondary bacterial infection. It appeared these differences were regional; dolphins coming ashore on Virginia Beach died in warmer waters heavily contaminated with opportunistic bacteria. Over 50% of the 21 species of potentially pathogenic bacteria isolated from 48 dolphins were of the genus Vibrio. These seemed to have been associated with some of the problems in skin and blood vessels that ultimately killed many of the animals but were not the primary cause of disease. The overwhelming nature of some of the infections, which probably arose in the lung, may have been related to immunoincompetence, the cause of which cannot be established. The depletion of lymphoid follicles in spleen, lymph nodes, and the intestine supports this suggestion.

Some dolphins also had viral infections. Eight had a skin condition characteristic of dolphin pox (Geraci et al. 1979), complete with suspicious inclusion bodies but in which no virus particles could be detected. In view of public sentiment expressed during the outbreak, it was comforting but not surprising to learn that none of the dolphins examined showed evidence of retroviruses, the group of viruses which is associated with Acquired Immune Deficiency Syndrome (AIDS) and whose counterparts in animals could have been a cause of reduced ability to fight normally harmless diseases. In any event, such viruses have a long latent period, and would not likely culminate in a single outbreak of disease. Dr. K. Somers is continuing to characterize the reovirus-like particles isolated from an ulcer on the palate of a dolphin. It is premature to comment on the serological titers to canine distemper virus, a morbillivirus, in six of 13 blood samples. Kennedy et al. (1988) have diagnosed morbillivirus infection and found distemper-like lesions in harbor porpoises (Phocoena phocoena) from the Irish Sea. We found no evidence of such infection nor was a morbillivirus detected using techniques suitable for its propagation. It is possible that the dolphins had been previously infected with a virus that escaped detection, or was no longer present at the time of the outbreak. A study must be undertaken to determine whether the virus or other antigen responsible for the serological reaction is widespread in dolphins and whether it is a pathogen. This calls for an examination of blood samples from a broad range of cetaceans, and an investigation into the nature of the antigen.

Geographic and temporal patterns of mortality also lacked the hallmark of infectious disease. During August 1987, at least 125 dolphins stranded dead along the Virginia coastline; nearly 50 came ashore in each of the months before and after. Others, according to fish-spotter pilot Mr. D. Thompson, were reported dead in small clusters at sea 18 miles from Cape May, NJ (August 21, 1987). To create such an overall pattern, an infectious agent would have had to be highly virulent -- causing acute disease across all ages and both sexes, spreading rapidly over a broad geographic range, and killing groups of animals without pause. Viruses and some bacteria introduced either by airborne transmission or through direct contact are capable of producing such havoc. Seals exposed on crowded rookeries have fallen victim to epizootics of influenza (Geraci et al. 1982), morbillivirus (Mahy et al. 1988, Osterhaus and Vedder 1988) and leptospirosis (Vedros et al. 1971). Yet, there is little to suggest that

these or other contagious organisms could spread as explosively among cetaceans. Dolphins are more dispersed in an environment which, unlike air, solid substrate or even a closed body of water, would not readily support the transmission of such agents.

The accumulating evidence led us to consider a point source contaminant as the cause of mortality. This was also a subject of public concern, as reflected by a train of media reports that sewage and toxic wastes were being discharged in the New York Bight and Delaware Bay areas. We approached the Environmental Protection Agency to obtain information on permitted and illegal dumping of municipal and industrial wastes off the mid-Atlantic states, and submitted tissues for heavy metal and organochlorine contaminant analysis.

Levels of contaminants in the dolphins' blubber were found to be among the highest recorded for a cetacean (Gaskin et al. 1971, 1983, Aguilar 1983, Tanabe et al. 1984, Martineau et al. 1987, Muir et al. 1988). Unfortunately, it is not possible to compare the levels with those in other T. truncatus as the only study on this species employed a different technique (King 1987). To ensure that the high values were not an artefact of our methodology, we analyzed blubber and liver samples from pilot and humpback whales, and harbor porpoises, for which published data exist. Results of PCB, DDE, and t-nonachlor analyses on the pilot whales agree closely with the recent findings of Muir (1988) for the same species. Residues in the blubber of humpback whales (DDE and PCB) are comparable to those reported by Taruski et al. (1975). Our DDE and PCB values in the harbor porpoise are similar to or lower than Gaskin's et al. (1971, 1983). The values in Tursiops stand unreservedly among the highest in cetaceans - a commentary on the state of eastern coastal waters.

High organochlorine levels in T. truncatus were not restricted to the stranded group; the captives had concentrations similar to those in all but the stranded mature males. The results from the beach-cast specimens obviously reflect the levels of contaminants in the nearshore environment, where the dolphins accumulate these substances. The residues occur in the blubber of captives perhaps because they are given contaminated food, or more likely because with a steady diet, they have no need to mobilize blubber fat which would deliver the compounds to liver for excretion. Under these stable conditions, the presence of organochlorines in blubber may not pose a risk. Free-ranging animals facing intermittent food supply, or mobilizing fat during lactation, migration or times of illness, release compounds from this depot into vital, perhaps more critical organs such as liver.

Considering the evidence that at least some of the dolphins were mobilizing PCBs from blubber to liver, it is conceivable that blood levels rose and were sustained long enough to exert an effect. One class of organochlorines, the polychlorinated biphenyls (PCBs), can be harmful following both acute and chronic exposure (Safe 1985). Typically affected are liver and skin, and nervous, reproductive, and immune systems (Safe 1985). Yet we cannot categorically relate any of the conditions observed in the dolphins to the known effects of these compounds because of vast differences in response within and between species. Furthermore, it is unlikely that contaminants were the key to the event. The timing of the outbreak would have required that these compounds be mobilized to

functionally toxic levels within a synchronized time-pulse. This is an unlikely scenario for substances which for decades have been a constant ingredient in their environment and body tissues, unless something else triggered their release by first debilitating the dolphins.

Biotoxins were considered to have this capability. The possibility was strengthened when saxitoxin, a neurotoxin produced by marine dinoflagellates, was found to be responsible for the deaths of 14 humpback whales, Megaptera novaeangliae, in early December 1987 and January 1988, in Cape Cod Bay (Geraci et al. submitted for publication). On the heels of that study, we analyzed liver samples from 17 dolphins that had died during the early, middle and late phases of the outbreak. There was no evidence of saxitoxin in these tissues.

By late summer 1988, some of the dolphin liver samples were reported to contain brevetoxin (PbTx), a lipid-soluble polyether toxin produced by the unarmored marine dinoflagellate Ptychodiscus brevis, Florida's red tide organism. The neurotoxin is extraordinarily potent, capable of generating effects in the nanomolar to picomolar concentration range in vivo (Baden, in press). When the analyses were completed in January, 1989, PbTx was found to be in the livers of eight of the 17 beached dolphins collected during the outbreak. No toxin was detected in any of the 17 controls, selected from dolphins that died in captivity, others in regions or at a time not related to the fatalities under investigation, and three that died during capture in October, 1987 (Table 6). A greater number of analyses would have added statistical weight to these findings. Yet the tests are time-consuming, and by this writing, 34 dolphin samples in addition to the fish specimens were all that could be processed. The pattern is nevertheless clear: 47% of the 17 diseased animals contained the toxin; all the rest did not.

Levels in dolphin liver ranged between 80-16,000 ng/g, and the calculated total amount in that organ was 0.08-14.7 mg. Assuming all the toxin was confined to liver, the total body burden would have been 2-290 μg/kg, comparable to or orders of magnitude higher than the 2.85 μg/kg level known to cause illness in man (McFarren et al. 1965). These values are conservative. Standard extraction procedures are only quantitative for one unaltered form of PbTx. Other forms that are covalently bound or otherwise modified were not considered. Nor is it reasonable to assume that all the toxin was in liver.

Signs of PbTx poisoning in fish and mammals are related to its action on the nervous system. Mice lose motor control, become paralyzed and die of respiratory arrest (Baden and Mende 1982). The site of action is the voltage-sensitive sodium channel in excitable membranes, where the toxin causes increased sodium flux with subsequent depolarization and persistent activation of excitable cells (Poli et al., in press). Death is rapid, and there are no reports of discernable histopathologic changes in acutely poisoned animals. Might this account for the presence of PbTx in a menhaden recently consumed by dolphin KDL-644 that showed no evidence of toxin in its liver?

Most of the dolphins did not die this way. They manifested an array of chronic disorders including fibrosis of liver and lung, adhesions of abdominal and thoracic viscera, and secondary microbial infections associated with immune suppression, as evidenced by histological changes in lymph nodes. We suggest

that sublethal exposure to PbTx precipitated the train of events leading to some or all of these chronic changes. PbTx promotes peripheral vasodilation (Poli et al., in press) and is cardiotoxic (Rodgers et al. 1984). As a toxic aerosol, or once absorbed, it disrupts neural control of respiration (Borison et al. 1980) and induces bronchoconstriction (Baden et al. 1982). Symptoms of poisoning in humans reflect the gastrointestinal and neurologic action of the toxin. They include nausea, vomiting, diarrhea, reversal of temperature sensation, ataxia, and numbness and tingling of extremities (Baden 1983). A dolphin so affected would likely stop eating, eventually exhaust its blubber reserve, and thereby lose its passive buoyancy and thermal shield. The stress associated with these changes alone could set the stage for infection by the ubiquitous opportunistic organisms that were isolated from the affected dolphins. Superimposed on this, any direct neurotoxic effect of PbTx would be particularly threatening to a diving mammal.

How were dolphins exposed to the toxin? Red tides in southeastern U.S. waters normally originate 20-75 km west of the central Florida coast in the Gulf of Mexico (Steidinger and Haddad 1981), and generally remain within the Gulf where they eventually dissipate. Occasionally, as in 1972, 1977, and 1980 (Roberts 1979, Steidinger and Baden 1984), they can be entrained and transported to the east coast of Florida by the Gulf Loop Current-Florida Current-Gulf Stream system. This happened in the fall of 1987, and resulted in the eventual closure of shellfish beds along the North Carolina coast; there also were reports of respiratory and eye irritation in fishermen and residents (Tester et al. in press). Yet the toxin was found in the livers of dolphins that beached in Virginia three months before that time. They must have encountered the organisms sometime and somewhere along their northerly migration route.

In February, 1987, a P. brevis bloom was 25 km from a point where Gulf waters are transported to the east coast. Drift bottle data (Williams et al. 1977) suggest that a fragment could have reached the east coast by spring of that year. The possibility exists that blooms had been occurring all summer in and adjacent to the Gulf Stream, and went undetected until a filament reached the North Carolina coast in October, 1987. Such blooms would have been difficult to detect at sea, as they are not easily seen from vessels and there would have been little in the way of toxic aerosols, which are generally produced by waves and surf action in shallow waters. Planktivorous fish might have consumed the cells offshore during their migration northward. And dolphins could have obtained the toxin by eating these fish or their predators. These conditions would have exposed dolphins both directly in water, and indirectly in food, to PbTx for an extended period, with effects manifested a short time later as they reached the mid-Atlantic coast.

Brevetoxin was recovered from three yellowfin menhaden, B. smithii, caught off Vero Beach, FL, in late February 1988, and one unidentified menhaden taken from the stomach of a dolphin that stranded near Cape Canaveral on January 12, 1988. The finding of brevetoxin in fish at that time and place suggests that there was a persistent, undetected bloom that kept the food-web contaminated through the winter. Alternatively, the bloom that had delivered the filament

to North Carolina in October 1987, had dissipated and left fish contaminated for at least three months. The first scenario challenges our understanding of the process of P. brevis blooms, the second of the dynamics of brevetoxin transfer in marine organisms.

In the fall of 1987, on their southerly migration, dolphins encountered the bloom off North Carolina. P. Tester (NOAA-NMFS Beaufort Laboratory, pers. comm.) observed dolphins surfacing in the blooms at that time. Three months later, and perhaps all along, they were feeding on contaminated fish. We believe that this second encounter with the toxin was responsible for the wave of stranded animals recovered along the Florida coast in the winter of 1987-1988; three of six dolphins examined had PbTx-2 in liver.

Levels of PbTx in the viscera of the live-caught menhaden translate to 200 μg of toxin per 500 g fish. Using this value, a dolphin feeding on menhaden at a rate of 10 kg each day, would consume 4 mg of PbTx. That is below the 6 mg/kg LD50 for mice, but if general toxicological dogma is applied, much lower doses would be required to incapacitate an animal as large as a dolphin. In fact, only 0.2 mg can cause illness in people.

Not all the dolphins were poisoned by eating fish. PbTx was found in the livers of three nursing calves. Dolphin WAM 295, with the highest concentration of PbTx in liver, was estimated to be less than 3 months of age. The toxin had to have been delivered in the milk, suggesting that like other lipid soluble residues, PbTx may be stored in fatty depots and mobilized along with fats as the animal draws on these reserves. There is no precedent for the finding of PbTx in milk, nor has this route of PbTx elimination been considered.

The circumstantial evidence suggests that PbTx is the most probable cause of the mortality. Contributing to the ultimate demise of the animals was a host of microbial and environmental factors. This is unlikely to have been the first time that dolphins have been exposed to the toxin. P. brevis blooms regularly occur on the Gulf coast of Florida. There they are restricted geographically in contrast to dolphins which move about freely. The chance of encounters is therefore reduced. They do occur, and at least one other associated mortality of dolphins has been reported (Gunter et al. 1948). Because there has been no search for biotoxins in stranded animals, other poisonings would have gone undiagnosed. One might also speculate that dolphins in the Gulf of Mexico have encountered blooms often enough to associate malaise with the ingestion of toxin-containing organisms or the aerosol, and thereafter avoid contact.

The episode along the east coast obviously required that the circumstances that delivered the organisms there be coupled with the presence of carrier-fishes situated in the path of migrating dolphins. The unparalleled scope of this event would suggest that all of these conditions have been met rarely, if at all, in the past. The summer of 1987 was unusual by any measure. In North Carolina, human poisoning from consumption of fish (Bonaventura and Bonaventura 1987) and shellfish (Tester et al. 1989) further attest to the unusual conditions that year.

The toxin in yellowfin menhaden has relevance to human health. Though not a fish that is commercially harvested, its southern range overlaps with related species of surface-feeding planktivores that are. In this case, the toxin was present in viscera and not the flesh, thus presenting no risk to humans consuming traditionally prepared fish, or the oils which are extracted under conditions that should destroy the toxin (Poli 1988). To establish whether a risk in fact exists, studies should be directed toward determining the uptake, distribution, persistence and transfer of PbTx in some representative commercially exploited species.

The discovery of PbTx in the dolphins and its previous circumstantial link to manatee deaths (O'Shea and Rathbun 1983) lead to a new generation of thought on factors contributing to natural mortality of marine mammals. Many questions will remain unanswered until directed studies are pursued. They must include: judicious examination of a representative sample of stranded marine mammals for biological toxins; studies on effects of chronic, sublethal exposure to PbTx; retrospective correlations between blooms and peak episodes of mortality; and determination of the environmental conditions that lead to the unusual event of 1987. Equally important is the need to resolve the growing question of whether contaminants at levels found in the dolphins might have affected their resilience and rendered them more susceptible either to the toxin or to the microorganisms that eventually brought them to their demise.

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Figure 1. Frequency distribution of the concentration of lipid extracted from blubber and liver of stranded bottlenose dolphins.

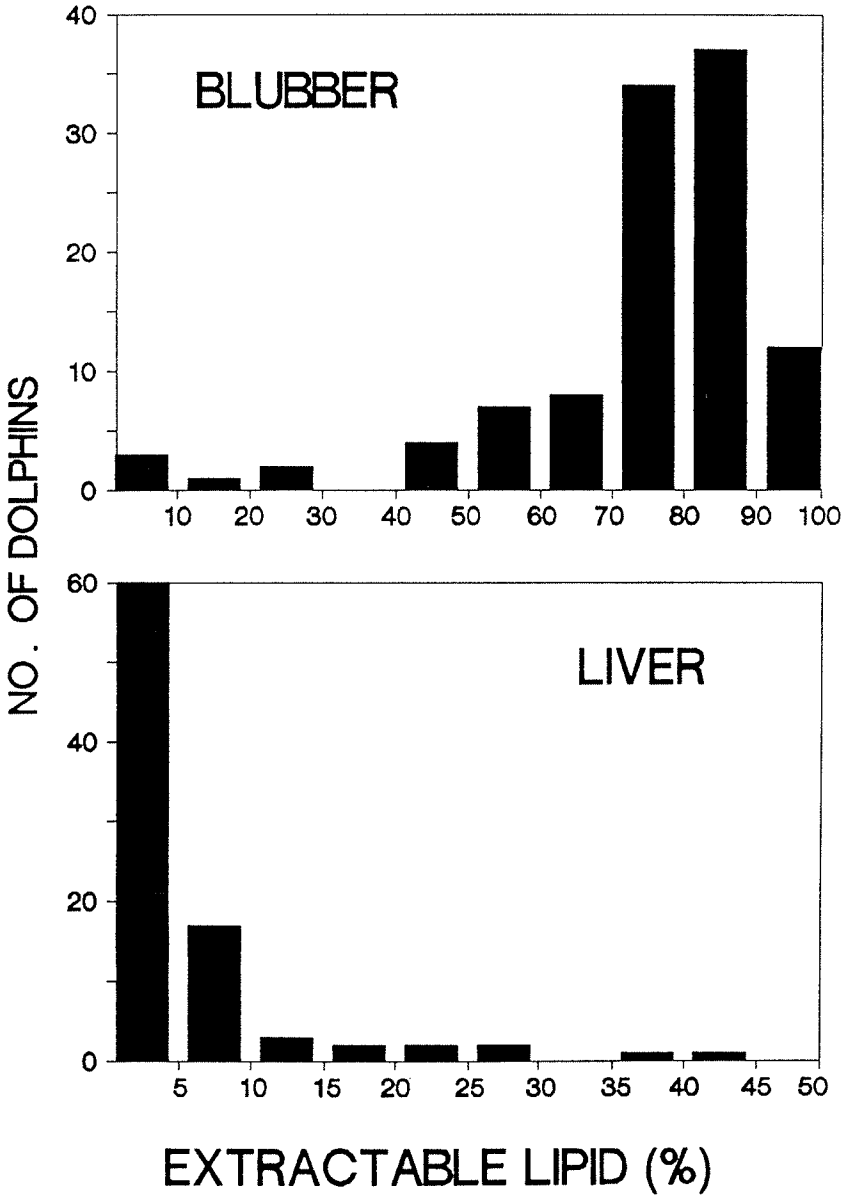
BLUBBER
LIVER
NO. OF DOLPHINS
EXTRACTABLE LIPID (%)
0
10
20
30
40
0
20
40
60
10 20 30 40 50 60 70 80 90 100
5 10 15 20 25 30 35 40 45 50

Figure 1

Figure 2. PCB concentrations in liver of stranded bottlenose dolphins, compared on the basis of lipid weight and wet weight as a function of lipid content in liver. None of the dolphins with liver lipid concentrations greater than 15% had PCB concentrations above 200 ppm.

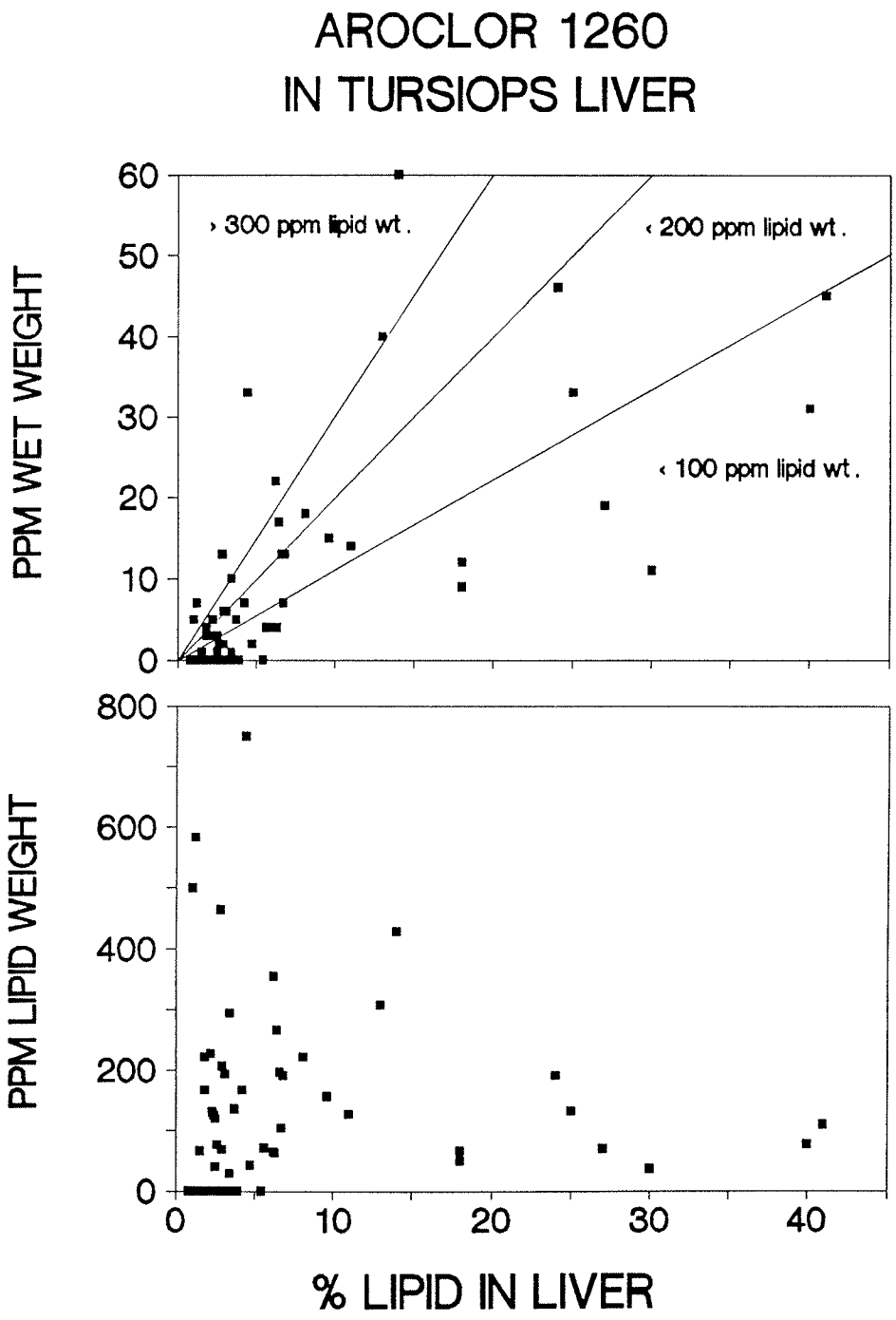
AROCLOR 1260
IN TURSIOPS LIVER
PPM WET WEIGHT
60
50
40
30
20
10
0
› 300 ppm lipid wt.
‹ 200 ppm lipid wt.
‹ 100 ppm lipid wt.
PPM LIPID WEIGHT
800
600
400
200
0
0
10
20
30
40
% LIPID IN LIVER

Figure 2

Figure 3. Comparison of the concentrations of three organochlorines in the liver and blubber of stranded bottlenose dolphins. Points lying above the line represent animals having greater concentrations in liver than in blubber, suggesting inability of the liver to clear the compounds.

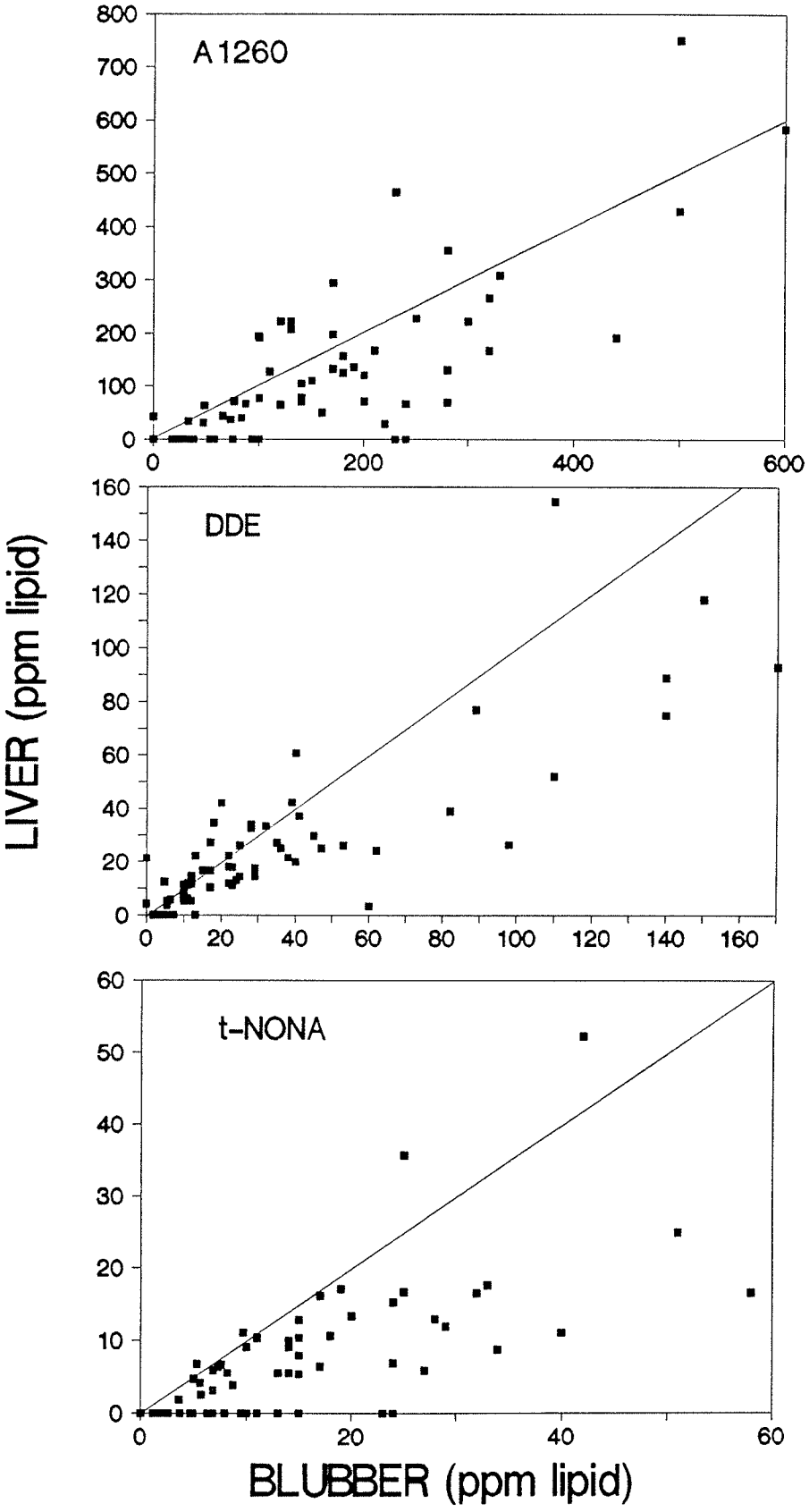
A1260
DDE
t-NONA
LIVER (ppm lipid)
BLUBBER (ppm lipid)
0
100
200
300
400
500
600
700
800
0
200
400
600
0
20
40
60
80
100
120
140
160
0
20
40
60
0
10
20
30
40
50
60

Figure 3

Table 1. Analytical disposition of 347 specimens collected during the 1987-88 mass mortality of bottlenose dolphins.

Analysis	No. of Dolphins	No. of Analyses
Partial necropsy	240	
Morphometric exam	61	
Complete necropsy	46	
Histopathology	95	2,660
Bacteriology	48	117
- Chlamydia	42	116
Virology	63	721
Toxicology		
- Organochlorine	75	1,456
- Heavy metals	68	1,079
Biotoxins		
- Water soluble	13	13
- Lipid soluble	34	34
Clinical pathology	26	1,106

Table 2. Tissues examined for evidence of viral infection.

Virus	Spleen	Liver	Kidney	Lung	Heart	Brain	Blood	Lymph node
Influenza A	7	9	7	9	4	3	1	1
Influenza B	7	9	7	9	4	3	1	12
Parainfluenza 1	7	9	7	9	4	3	1	12
Parainfluenza 3	7	9	7	9	4	3	1	12
Herpes 1	7	9	7	9	4	3	1	12
Herpes 2	7	9	7	9	4	3	1	12
Varicella- Zoster	7	9	7	9	4	3	1	12
Adenovirus	7	9	7	9	4	3	1	12
Papovirus	7	9	7	9	4	3	1	1
Bovine Leucosis	1				4		11	4
Bluetongue	1			4			11	4
Equine Infectious Anemia		1			4		9	4
Equine Rhinopneumonitis	1			3			9	4
Equine Herpes 2							13	
Coital Exanthema							15	
Ovine Progressive Pneumonia	1			4			9	4
Vesicular Stomatitis	1			4			9	4
Parvovirus							1	
Enterovirus							1	
AIDS virus							17	
Retrovirus				4			11	
Contagious Ecthema				3			11	

Table 3: Gross necropsy findings in Tursiops truncatus. Observations reported in relation to the number of animals in which each organ system was examined.

OBSERVATION	OCCURRENCE				
	OVERALL	MALE	FEMALE	MATURE	IMMATURE
lymph nodes					
necrosis	5/51	3/27	2/24	1/14	4/37
fibrosis	11/51	6/27	5/24	6/14[a]	5/37[a]
lymphadenitis	35/51	16/27	19/24	10/14	25/37
integument					
tattoo (pox)	6/119*	2/52	4/56	1/36	5/83
pock (craters, fissures)	6/119	3/52	3/56	5/36[b]	1/83[b]
ulcers	27/119	12/52	14/56	6/36	21/83
blisters (vesicles)	4/119	3/52	1/56	0/36	4/83
vascular lesions	6/119	1/52	5/56	2/36	4/83
necrosis	90/119	50/52	39/56	21/36	69/83
abdominal cavity					
adhesions	21/68	12/37	8/31	7/12	14/56
fluid - clear	8/68	6/37	2/31	3/12	5/56
fluid - sero-sanguineous	30/68	16/37	14/31	5/12	25/56
serosal fibrosis	7/68	3/37	4/31	3/12	4/56
thoracic cavity					
adhesions	14/51	9/30	5/20	5/12	9/39
fluid - clear	25/51	10/30	14/20	5/12	20/39
fluid - sero-sanguineous	34/51	24/30	10/20	7/12	27/39
pleural fibrosis	12/51	5/30	7/20	2/12	10/39
liver					
fibrosis	29/63	13/31	16/32	15/19	14/44
fatty (pale, yellow)	21/63	10/31	11/32	4/19	17/44
congestion	17/63	9/31	8/32	4/19	13/44
capsular fibrosis	14/63	8/31	6/32	5/19	9/44
degeneration	9/63	5/31	4/32	1/19	8/44
lung, pleura					
necrosis	40/113	20/55	20/58	7/27	33/86
edema	38/113	21/55	17/58	10/27	28/86
congestion	37/113	18/55	19/58	10/27	27/86
hemorrhage	11/113	7/55	4/58	2/27	9/86
fibrosis	18/113	7/55	11/58	5/27	13/86
parasitic pneumonia	22/113	12/55	10/58	3/27	19/86
interstitial pneumonia	40/113	16/55	24/58	11/27	29/86
bronchitis	6/113	1/55	5/58	1/27	5/86
pleura	26/113	13/55	13/58	8/27	18/86

[a] significantly different $p < 0.05$
[b] significantly different $p < 0.01$
* totals include 1 animal not sexed

Table 4: Histopathologic findings in Tursiops

OBSERVATION	OCCURRENCE				
	OVERALL	MALE	FEMALE	MATURE	IMMATURE
lymph node					
lymphadenitis	18/62*	10/28	8/33	10/20[a]	8/42[a]
follicle depletion	38/62	20/28	18/33	11/20	27/42
liver					
capsular fibrosis	16/67*	7/30	9/35	10/24[a]	6/43[a]
biliary fibrosis	7/67	5/30	2/35	4/24	3/43
parenchymal fibrosis	25/67	10/30	15/35	14/24[a]	11/43[a]
hepatitis	8/67	3/30	5/35	3/24	5/43
integument					
parakeratosis	15/62*	4/24	11/36	6/24	9/38
inclusions	8/62	5/24	3/36	3/24	5/38
ulcers (with dermatitis)	28/62	11/24	16/36	17/24[a]	11/38[a]
ulcers (without dermatitis)	1/62	1/24	0/36	0/24	1/38
lung					
pleura					
inflammation	5/77*	2/34	3/34	2/27	3/50
fibrosis	40/77	20/34	20/43	20/27[a]	20/50[a]
parenchyma					
mycotic infection	16/77	6/34	10/43	4/27	12/50
bacterial infection	10/77	4/34	6/43	5/27	5/50
parasitic infection	15/77	7/34	8/43	2/27	13/50
fibrosis	30/77	15/34	15/43	13/27	17/50
edema/congestion	14/77	6/34	8/43	5/27	9/50
bronchi/bronchioles					
desquamation	56/77	24/34	32/43	19/27	37/50
necrosis	10/77	6/34	4/43	2/27	8/50
inflammation	14/77	2/34	12/43	4/27[a]	10/50[a]
exudate	35/77	16/34	19/43	13/27	22/50
aspiration	10/77	3/34	7/43	1/27	9/50
vessel inflammation	2/77	1/34	1/43	1/27	2/50
heart					
myocardial fibrosis	16/54	6/23	10/31	7/16	9/38

[a] significantly different $p < 0.05$
* totals include animals not sexed

Table 5. Bacteria isolated from the tissues of 48 bottlenose dolphins examined during the mass mortality.

	Liver	Spleen	Lung	Lymph Node	Blood	Urine	Blubber	Abdom. Fluid	Kidney	Brain
No. of specimens	19	15	27	15	15	5	2	5	11	3
Vibrio sp.	12	9	26	13	8	2	2	1	7	3
V. parahaemolyticus	6	6	4	1	4		1		4	
V. damsela	2	1	6	1	1				1	1
V. alginolyticus	2	1	11	9	2		1		2	1
V. harveyi	2		1	2	1				4	
V. vulnificus	1		1			1		1		
V. nereis						1				
Edwardsiella sp.	8	6	10	3	1	1	1	3	3	
E. tarda	8	5	10	3	1	1	1	2	3	
E. hoshinae								1		
Alteromonas sp.	1	1	7	1	1	1	2	1		
A. putrefaciens	1	1	6	1	2	1	2	1		
Pseudomonas putrefaciens	1	1	2		2		1		1	
Enterobacter cloacae	1	1		1				1		
Acinetobacter lwoffi	1	1				1	1			
Streptococcus sp.	6		1	1		1			3	1
S. equisimilus	2	2	1							2
Escherichia coli	2	1	1	1					1	
Staphylococcus sp.	1	1	1	1	3		1			
Proteus sp.	1	1				1				
Morganella morganii		1								

Table 6: Results of brevetoxin analysis in dolphin liver samples.

SAMPLE	BIOASSAYS 1st	2nd	3rd	HPLC	CONC ng/g
Stranded, Virginia, Aug. 1987					
WAM 239	+	+	+	+	93
WAM 231	+	+	+	+	83
WAM 226	+	+	+	*	
WAM 214	+	+	+	**	
WAM 219	+	+	+	**	
JGM 448	+	-			
Stranded, Virginia, Sept.-Oct. 1987					
WAM 295	+	+	+	+	15820
WAM 280	+	+	+	+	14530
WAM 296	+	+	+	+	1851
WAM 282	+	+	+	**	
CWP 273	+	-			
Stranded, Florida, Jan.-Feb. 1988					
S-88-TT-51	+	+	+	+	14700
S-88-TT-57	+	+	+	+	310
S-88-TT-01	+	+	+	+	155
S-88-TT-11	+	+	+	*	
K 644	+	+	+	*	
SS-88-TT-04	+	+	+	**	
Died during capture, VA Beach, Oct. 1987					
VB-87-004	+	+	+	*	
VB-87-014	+	+	-		
VB-87-009	+	+	-		
Stranded - Texas, 1987-1988					
C 552	+	+	+	*	
C 391	+	-			
C 575	+	-			
Stranded mid-Atlantic Coast, Aug.-Nov. 1988					
WAM 331	+	+	-		
WAM 336	+	+	-		
WAM 340	+	+	-		
WAM 332	+	+	-		
WAM 335	+	+	-		
WAM 339	+	-			
Captive Tursiops					
MH82222 L21	+	+	-		
MH7408 L22	+	-			
MH7516	-				
MH79179	-				
Stranded - Cape Cod 1983					
MH83216	-				

* peak present but did not comigrate with standard
** no peak suggestive of PbTx

Table 7. Chlorinated hydrocarbon residues (as ppm lipid weight) in blubber from bottlenose dolphins recovered during the mass mortality. Samples from captive dolphins, pilot whales and harbour porpoise were analyzed concurrently for comparison.

		Aroclor 1260		DDE		Trans-Nonachlor		% Lipid	
SPECIMEN	N	x ± SD	RANGE	x ± SD	RANGE	x ± SD	RANGE	x ± SD	RANGE
Tursiops	56	181.6 ± 141.4	13-620	39.5 ± 44.7	3-200	14.6 ± 12.0	1-58	78.3 ± 10.0	50-99
Imm. Female	18	145.1 ± 126.6	29-500	28.6 ± 37.1	6-170	15.3 ± 12.1	1-51	81.0 ± 7.7	63-96
Mat. Female	9	122.8 ± 96.5	18-280	14.2 ± 14.9	3-53	7.4 ± 8.4	1-28	73.3 ± 9.0	58-86
Imm. Male	22	202.3 ± 139.5	33-600	36.6 ± 31.6	8-140	16.8 ± 13.3	1-58	79.4 ± 9.9	54-92
Mat. Male	6	328.3 ± 140.9	190-620	114.5 ± 49.0	45-200	20.7 ± 5.3	13-28	69.1 ± 9.3[a]	50-79
Captive	3	177.7 ± 110.1	33-300	106.0 ± 55.3	28-150	18.4 ± 10.9	5-32	78.7 ± 4.5	75-85
Globicephala melaena	11	26 ± 20	10-69	22.1 ± 19.2	6-70	6.6 ± 3.8	4-18	82.5 ± 6.0	72-94
Phocoena phocoena	8	24 ± 6	15-33	8.2 ± 2.9	5-14	7.8 ± 2.6	5-12	89.3 ± 5.4	82-95
Megaptera novaeangliae	8	13 ± 12	6-44	4.5 ± 4.9	1-17	1.5 ± 2.0	0.2-7	73.0 ± 9.8	50-89

Table 8. Chlorinated hydrocarbon residues (as ppm lipid weight) in liver from bottlenose dolphins recovered during the mass mortality. Samples from captive dolphins, pilot whales and harbour porpoise were analyzed concurrently for comparison.

		Aroclor 1260		DDE		trans-Nonachlor		% Lipid	
SPECIMEN	N	x ± SD	RANGE	x ± SD	RANGE	x ± SD	RANGE	x ± SD	RANGE
Tursiops	53	145.7 ± 161.6	0-750	24.5 ± 26.5	0-155	8.1 ± 9.5	0-52	7.4 ± 9.5	0.8-41
Imm. Female	21	115.1 ± 96.7	0-429	20.4 ± 19.4	3-93	7.6 ± 6.4	0-25	11.2 ± 11.7	0.9-41
Mat. Female	11	72.4 ± 101.6	0-294	8.2 ± 8.9	0-26	2.5 ± 4.4	0-13	2.2 ± 0.8	0.8-3
Imm. Male	17	205.2 ± 214.1	0-750	35.4 ± 34.5	7-155	12.4 ± 13.2	0-52	6.7 ± 8.3	0.9-30
Mat. Male	4	254.4 ± 165.6	75-500	44.0 ± 19.4	30-77	7.4 ± 5.6	0-15	5.2 ± 4.6	1.0-13
Captive	3	109.2 ± 81.4	34-222	80.2 ± 34.8	34-118	11.4 ± 4.1	7-17	3.5 ± 1.8	1.8-5.9
Globicephala melaena	11	not detected		5.3 ± 10.8	0-38	1.6 ± 4.4	0-15	2.5 ± 1.5	1.0-5.7
Phocoena phocoena	9	46.2 ± 38.2	0-111	5.1 ± 3.8	0-11	3.8 ± 3.2	0-8.7	3.6 ± 1.6	1.6-6.2

Table 9. Chlorinated hydrocarbon residues (as ppm wet weight) in liver from bottlenose dolphins recovered during the mass mortality. Samples from captive dolphins, pilot whales and harbour porpoise were analyzed concurrently for comparison.

SPECIMEN	N	Aroclor 1260 x ± SD	RANGE	DDE x ± SD	RANGE	Trans-Nonachlor x ± SD	RANGE	% Moisture x ± SD	RANGE
Tursiops	53	10.3 ± 13.7	0-60	1.7 ± 2.6	0-13	0.8 ± 1.2	0-5.5	71.6 ± 7.7	43-81
Imm. Female	21	14.2 ± 17.4	0-60	2.4 ± 3.3	0.2-13	1.2 ± 1.7	0-5.5	67.9 ± 9.2	43-79
Mat. Female	11	2.0 ± 1.0	0-10	0.2 ± 0.2	0-0.6	0.1 ± 0.1	0-0.3	76.5 ± 2.2	72-81
Imm. Male	17	10.2 ± 9.1	0-33	1.7 ± 1.6	0.1-6.8	0.8 ± 0.8	0-2.7	72.5 ± 6.6	55-30
Mat. Male	4	13.0 ± 15.6	2-40	3.1 ± 4.0	0.3-10	0.6 ± 0.8	0-2.0	73.9 ± 2.2	70-76
Captive	3	2.7 ± 0.9	2-4	2.3 ± 0.7	1.6-3.3	0.3 ± 0.05	0-0.4	74.7 ± 1.2	73-76
Globicephala melaena	11	not detected		0.14 ± 0.23	0-0.8	0.03 ± 0.06	0-0.2	74.1 ± 4.5	63-78
Phocoena phocoena	9	1.8 ± 1.5	0-4	0.2 ± 0.16	0-0.5	0.14 ± 0.13	0-0.4	72.6 ± 2.3	71-78

Table 10. Statistical comparison [a] of organochlorine residues in blubber of stranded Tursiops.

	Arochlor 1260		
	Immature Males	Immature Females	Mature Females
Mature Males	*	**	**
Immature Males	-	n.s.	n.s.
Immature Females	-	-	n.s.

	DDE		
	Immature Males	Immature Females	Mature Females
Mature Males	**	**	**
Immature Males	-	n.s.	n.s.
Immature Females	-	-	n.s.

	trans-Nonachlor		
	Immature Males	Immature Females	Mature Females
Mature Males	n.s.	n.s.	*
Immature Males	-	n.s.	n.s.
Immature Females	-	-	n.s.

[a] comparisons made using Newman-Keuls ANOVA. n.s. - not significant; * , ** - $p < 0.05, 0.01$.

Table 11. Heavy metal analysis of liver collected from bottlenosed dolphins sampled during the mass mortality. Specimens from captive dolphins, pilot whales, and harbor porpoises were analyzed concurrently for comparison. Data expressed as ppm wet weight.

Specimen	N	Copper		Zinc		Lead	
		x + SD	RANGE	x + SD	RANGE	x + SD	RANGE
Tursiops	59	8.3 ± 5.7	0.08-28	76 ± 43	16-210	0.23 ± 0.67	0-3.1
Imm.Female	22	8.9 ± 6.6	1.4-28	89 ± 33	25-170	0.14 ± 0.43	0-1.6
Mat.Female	14	8.5 ± 5.8	2.6-23	39 ± 19	22-85	0.11 ± 0.41	0-1.6
Imm.Male	18	6.9 ± 4.3	0.1-17	92 ± 44	16-210	0.33 ± 0.77	0-2.6
Mat.Male	5	10 ± 5	3-15	68 ± 53	25-170	0.62 ± 1.24	0-3.1
Captive	3	15.5 ± 8.6	6.4-27	58 ± 12	42-68	1.7 ± 1.55	0-4.0
Globicephala melaena	11	11.8 ± 5.6	4.5-23	78 ± 54	42-240	1.2 ± 1.5	0-4.1
Phocoena phocoena	9	13 ± 8.4	5.1-31	64 ± 33	25-145	0.17 ± 0.47	0-1.5

Table 11, cont'd. Heavy metal analysis of liver collected from bottlenosed dolphins sampled during the mass mortality. Specimens from captive dolphins, pilot whales, and harbor porpoises were analyzed concurrently for comparison. Data expressed as ppm wet weight.

Specimen	N	Cadmium		Selenium		Mercury	
		x + SD	RANGE	x + SD	RANGE	x + SD	RANGE
Tursiops	59	not detected		9 ± 12	0-51	22 ± 27	0-110
Imm.Female	21	not detected		2 ± 4	0-12	5 ± 7	0-25
Mat.Female	14	not detected		22 ± 14	5-51	55 ± 26	21-10
Imm.Male	18	not detected		4 ± 7	0-24	12 ± 19	0-75
Mat.Male	5	not detected		15.9 ± 8	5.3-29	32 ± 16	13-59
Captive	3	not detected		not detected		12 ± 11	2-28
Globicephala melaena	11	15 ± 11	0-35	12 ± 13	0-37	29 ± 36	0-105
Phocoena phocoena	9	0.5 ± 1.3	0-4.2	not detected		1.6 ± 1.6	0-3.8

Appendix 1. Data on Tursiops truncatus examined during the clinical investigation.

Dolphin ID	Stranding Date	Location	Age[1]	Length (cm)	Sex	CHC[2]	Heavy Metals	Necropsy[3]	Histo-Pathol	Virol.	Micro-biol.
S-87-TT-02	01-07-87	Ormond Beach FL	-[4]	122	F	-	-	P	-	-	-
S-87-TT-04	01-10-87	Painter's Hill FL	-	201	F	-	-	P	-	-	-
S-87-SF-06	02-03-87	Pte Verda Beach FL	-	217	M	-	-	P	-	-	-
S-87-TT-07	03-11-87	Pte Verda Beach FL	-	122	M	-	-	P	-	-	-
S-87-TT-08	03-11-87	South Ponte Verda Beach FL	-	122	M	-	-	P	-	-	-
S-87-TT-09	04-23-87	South Ponte Verda FL	-	167	-	-	-	P	-	-	-
WAM-139	05-15-87	Norfolk VA	I	205	F	-	-	P	-	-	-
WAM-144	05-21-87	Virginia Beach VA	M	247	F	-	-	P	-	-	-
WAM-147	05-26-87	Ship Shoal Is. VS	I	115	F	-	-	P	-	-	-
WAM-148	05-26-87	Hog Island VA	-	112	M	-	-	P	-	-	-

[1]Dolphins are classified as mature (M) or immature (I) based on examination of reproductive organs and/or vertebral epiphyses. Age data expressed as year class, determined by tooth layer counts (S. Hersh, NMFS); p - perinate (<3 months).

[2]Chlorinated hydrocarbon analyses.

[3]C -complete necropsy; P - partial necropsy; ND - no data; LC - live capture.

4(+) = analysis performed; (-) = no analysis performed.

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-142	05-29-87	Little Creek VA	-	108	F	-	-	-	-	-	-
JGM-446	06-06-87	Seaford VA	-	252	M	-	-	P	-	-	-
S-87-TT-12	06-07-87	Staug Beach FL	-	202	F	-	-	P	-	-	-
WAM-151	06-08-87	Virginia Beach VA	-	102	F	-	-	P	-	-	-
WAM-152	06-08-87	Frisco NC	M	254	F	-	-	P	-	-	-
WAM-153	06-08-87	Assateague Island MD	I	214	M	-	-	P	-	-	-
WAM-155	06-17-87	Cape Charles VA	-	215	M	-	-	-	-	-	-
WAM-154	06-18-87	Cape Charles VA	-	205	M	-	-	-	-	-	-
WAM-161	06-25-87	Assateague Island MD	-	105	M	-	-	P	-	-	-
WAM-160	06-27-87	Assateague Island MD	I	228	M	-	-	P	-	-	-
WAM-158	06-28-87	Cape Charles VA	-	230	M	-	-	-	-	-	-
WAM-159	06-28-87	Cape Charles VA	-	238	M	-	-	P	-	-	-
WAM-156	06-29-87	Penney's Hill VA	-	246	-	-	-	P	-	-	-
WAM-157	06-29-87	False Cape VA	-	249	F	-	-	P	-	-	-
WAM-166	07-02-87	Bellehaven VA	-	167	F	-	-	-	-	-	-
WAM-163	07-06-87	Fort Story VA	-	210	F	-	-	-	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-165	07-06-87	Mathews County VA	23	281	M	-	-	P	-	-	-
WAM-162	07-07-87	Ocean City MD	M	250	F	-	-	P	-	-	-
WAM-164	07-08-87	Hampton VA	8	241	M	-	-	P	-	-	-
WAM-167	07-08-87	Assateague Island MD	-	221	F	-	-	P	-	-	-
WAM-168	07-11-87	Virginia Beach VA	-	202	M	-	-	P	-	-	-
WAM-169	07-11-87	Virginia Beach VA	-	199	F	-	-	P	-	-	-
WAM-170	07-11-87	Haven Beach VA	14	270	M	-	-	P	-	-	-
WAM-190	07-12-87	Assateague Island MD	1	175	F	-	-	-	-	-	-
WAM-171	07-13-87	Seaford VA	-	219	M	-	-	P	-	-	-
WAM-172	07-13-87	Virginia Beach VA	I	-	-	-	-	P	-	-	-
WAM-173	07-16-87	Sandbridge VA	I	183	F	-	-	-	-	-	-
WAM-174	07-16-87	Sandbridge VA	16	269	M	-	-	-	-	-	-
CWP-249	07-17-87	Worcester MD	7	258	M	-	-	-	-	-	-
WAM-175	07-17-87	Lynn Haven Inlet VA	9	240	F	-	-	P	-	-	-
WAM-179	07-17-87	False Cape VA	-	220	F	-	-	P	-	-	-
CWP-251	07-18-87	Milford Haven VA	3	222	M	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-188	07-18-87	Hampton VA	-	245	F	-	-	-	-	-	-
WAM-176	07-19-87	Virginia Beach VA	11	241	F	-	-	P	-	-	-
WAM-178	07-21-87	Hampton VA	9	267	M	-	-	-	-	-	-
WAM-180	07-21-87	Virginia Beach VA	I	220	-	-	-	P	-	-	-
WAM-181	07-21-87	Norfolk VA	21	274	M	-	-	-	-	-	-
WAM-182	07-21-87	Norfolk VA	11	255	M	-	-	-	-	-	-
WAM-184	07-22-87	False Cape VA	-	-	-	-	-	P	-	-	-
JGM-448	07-23-87	Assateague Island MD	5	238	M	-	-	P	-	-	-
WAM-186	07-23-87	Hampton VA	I	211	M	-	-	P	-	-	-
WAM-187	07-24-87	Hampton VA	9	254	F	-	-	-	-	-	-
WAM-189	07-28-87	Virginia Beach VA	-	254	M	-	-	P	-	-	-
WAM-191	07-29-87	Little Creek VA	4	233	F	-	-	P	-	-	-
WAM-192	07-29-87	Virginia Beach VA	2	202	M	-	-	P	-	-	-
WAM-194	07-29-87	False Cape VA	-	-	-	-	-	-	-	-	-
WAM-195	07-29-87	Ocean City MD	-	183	-	-	-	P	-	-	-
WAM-193	07-30-87	False Cape VA	14	284	M	-	-	P	-	-	-
WAM-196	07-30-87	Assateauge Island MD	I	216	-	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-197	07-31-87	Croaton Beach VA	23	270	F	-	-	P	-	-	-
WAM-198	07-31-87	Deacroke Island	14	270	M	-	-	P	-	-	-
CWP-252	08-02-87	Worcester MD	17+	259	F	-	-	P	-	-	-
WAM-199	08-02-87	Norfolk VA	8	242	F	-	-	-	-	-	-
WAM-200	08-02-87	Virginia Beach VA	4	237	M	-	-	P	-	-	-
WAM-201	08-02-87	Virginia Beach VA	2	212	M	-	-	P	-	-	-
WAM-203	08-02-87	Gwynn Island VA	I	-	-	-	-	P	-	-	-
WAM-213	08-02-87	Little Creek VA	4	222	F	-	-	-	-	-	-
WAM-202	08-03-87	Virginia Beach VA	1	206	M	-	-	P	-	-	-
CWP-253	08-04-87	Hampton VA	4	233	F	-	-	P	-	-	-
CWP-254	08-04-87	Hampton VA	8	236	F	-	-	-	-	-	-
CWP-255	08-04-87	Virginia Beach VA	I	203	F	-	-	P	-	-	-
CWP-256	08-04-87	Virginia Beach VA	4	223	M	-	-	P	-	-	-
WAM-204	08-04-87	Hampton VA	-	140	M	-	-	P	-	-	-
S-87-TT-14	08-06-87	Mayport FL	-	272	M	-	-	P	-	-	-
WAM-205	08-06-87	Damneck VA	I	198	M	-	-	C	-	-	-
WAM-206	08-06-87	Virginia Beach VA	13	242	M	-	-	C	+	+	+
WAM-207	08-06-87	Ocean Park VA	3	214	M	-	-	C	+	+	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-233	08-06-87	Back Bay VA	M	260	-	-	-	P	-	-	-
WAM-183	08-07-87	Norfolk VA	1p	154	M	-	-	-	-	-	-
WAM-208	08-07-87	Damneck VA	20+	303	M	-	-	P	+	+	+
WAM-216	08-07-87	Croatan Beach VA	-	-	-	-	-	-	-	-	-
WAM-217	08-07-87	Virginia Beach VA	4	241	F	-	-	-	-	-	-
S-87-TT-15	08-08-87	Mayport FL	M	270	M	-	-	P	-	-	-
WAM-209	08-08-87	Virginia Beach VA	1	188	F	+	+	C	+	+	+
WAM-210	08-08-87	Sandbridge VA	1p	159	F	+	+	C	+	+	+
WAM-211	08-08-87	Little Creek VA	5	197	F	-	-	P	-	-	-
WAM-212	08-08-87	Little Creek VA	-	164	F	-	-	P	-	-	-
WAM-214	08-08-87	Virginia Beach VA	-	242	F	-	-	C	+	+	+
WAM-215	08-09-87	Virginia Beach VA	-	240	F	-	-	P	-	-	-
WAM-218	08-09-87	Cape Henry VA	1p	148	M	-	-	P	-	-	-
VA-1	08-10-87	Virginia	I	-	-	+	+	-	-	+	+
WAM-219	08-10-87	Virginia Beach VA	-	134	M	-	-	P	-	-	-
70-87	08-11-87	Avalon NJ	-	267	F	-	-	-	+	+	+
72-87	08-11-87	Island Beach NJ	-	274	M	-	-	-	+	+	+
74-87	08-11-87	Long Beach Isl NJ	-	290	M	-	-	-	+	+	+

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
76-87	08-11-87	Island Beach NJ	-	224	M	+	+	-	+	+	+
CWP-260	08-11-87	Virginia Beach VA	6	239	F	-	-	P	-	-	-
DAP-011	08-11-87	Seashore State Park VA	1p	155	M	-	-	P	-	-	-
DAP-013	08-11-87	Lynn Haven Inl VA	4	232	M	-	-	P	-	-	-
WAM-220	08-11-87	Virginia Beach VA	1	220	-	+	-	P	+	-	-
WAM-221	08-11-87	Virginia Beach VA	1	184	F	-	-	P	-	-	-
WAM-222	08-11-87	Virginia Beach VA	-	140	F	-	-	P	-	-	-
WAM-223	08-11-87	Seashore State Park VA	2	187	F	-	-	P	-	-	-
WAM-234	08-11-87	Back Bay VA	I	199	M	-	-	-	-	-	-
CWP-257	08-12-87	Virginia Beach VA	1p	143	F	-	-	P	-	-	-
CWP-258	08-12-87	Virginia Beach VA	1	160	-	-	-	P	-	-	-
CWP-259	08-12-87	Virginia Beach VA	5	229	F	-	-	-	-	-	-
WAM-224	08-12-87	Seashore State Park VA	1	177	F	-	+	P	-	-	-
WAM-225	08-12-87	Little Creek VA	8	250	M	+	-	C	-	-	-
WAM-226	08-13-87	Virginia Beach VA	2	243	F	+	+	C	+	+	+
WAM-227	08-13-87	Virginia Beach VA	7	212	M	+	-	C	+	+	+

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-228	08-13-87	Chesapeake Beach VA	13	252	F	-	-	P	-	-	-
WAM-229	08-13-87	Fort Story VA	-	181	F	-	-	P	-	-	-
CWP-261	08-14-87	Virginia Beach VA	1	194	M	-	-	C	-	-	-
CWP-262	08-14-87	Virginia Beach VA	21+	248	M	-	-	P	-	-	-
S-87-TT-16	08-14-87	St. Augustine Beach FL	-	188	-	-	-	P	-	-	-
WAM-230	08-14-87	Virginia Beach VA	1p	143	F	+	+	C	+	+	+
WAM-231	08-14-87	Chesapeake Beach VA	I	240	F	+	+	C	+	+	+
WAM-232	08-14-87	Chesapeake Beach VA	16+	260	F	+	+	C	+	-	+
WAM-237	08-14-87	Portsmouth VA	M	232	F	-	-	P	-	-	-
85-87	08-15-87	Brigantine NJ	-	-	-	-	-	-	-	+	+
CWP-263	08-15-87	Virginia Beach VA	4	251	M	-	-	C	+	+	+
CWP-264	08-15-87	Virginia Beach VA	2	205	F	+	+	C	+	+	+
CWP-265	08-15-87	Virginia Beach VA	1p	134	F	-	-	P	-	-	-
CWP-266	08-15-87	Norfolk VA	12	285	M	-	-	C	+	-	-
WAM-235	08-15-87	Hampton VA	10	257	M	-	-	C	+	-	-
87-01	08-16-87	Virginia Beach VA	-	-	-	-	-	LC	-	-	+

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
87-02	08-16-87	Virginia Beach VA	-	-	-	-	-	LC	-	-	+
87-03	08-16-87	Virginia Beach VA	-	-	-	-	-	LC	-	-	+
WAM-236	08-16-87	Virginia Beach VA	I	220	F	-	-	C	+	-	+
S-87-TT-17	08-16-87	Ponce Inlet FL	-	137	M	-	-	P	-	-	-
DAP-014	08-20-87	Virginia Beach VA	7	226	M	-	-	P	-	-	-
WAM-238	08-20-87	Little Creek VA	1p	144	F	-	-	P	-	-	-
WAM-239	08-20-87	Sandridge VA	I	143	F	+	+	C	+	+	+
WAM-243	08-20-87	Back Bay VA	-	-	-	-	-	-	-	-	-
CWP-267	08-21-87	Virginia Beach VA	1	201	M	-	-	C	+	-	-
CWP-269	08-21-87	Seashore State Park VA	1	212	M	-	-	P	-	-	-
WAM-240	08-21-87	Wash Woods VA	1	186	F	-	-	P	-	-	-
WAM-241	08-21-87	North Carolina	3	227	F	-	-	P	-	-	-
WAM-242	08-21-87	False Cape VA	5	255	-	-	-	P	-	-	-
S-87-SF-18	08-22-87	Ponte Vedra FL	-	231	M	-	-	P	-	-	-
104-87	08-23-87	Townsend Inlet NJ	-	253	M	-	-	-	-	+	+
WAM-244	08-23-87	Virginia Beach VA	-	-	-	-	-	-	-	-	-
WAM-245	08-23-87	Chesapeake Bay VA	I	197	F	-	-	C	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-246	08-23-87	Virginia Beach VA	1p	115	F	-	-	C	+	-	-
WAM-247	08-24-87	Sandbridge VA	1	167	M	+	+	C	+	+	+
WAM-249	08-24-87	False Cape VA	-	-	-	-	-	-	-	-	-
WAM-250	08-24-87	False Cape VA	2	207	F	-	-	P	-	-	-
S-87-TT-19	08-26-87	Flagler Beach FL	-	155	F	-	-	P	-	-	-
WAM-251	08-26-87	Sandbridge VA	I	232	M	+	+	C	+	+	+
WAM-252	08-29-87	Black Croatan Beach VA	1p	151	M	+	+	C	+	+	+
WAM-253	08-29-87	Fort Story VA	22	281	M	+	+	C	+	+	+
WAM-254	08-30-87	Damneck VA	2	194	M	+	+	C	+	-	-
WAM-255	08-30-87	Virginia Beach VA	I	190	F	+	+	C	+	+	+
JGM-450	08-31-87	Virginia Beach VA	20	260	F	-	-	C	+	+	+
WAM-257	09-01-87	Fort Story VA	1	159	F	-	-	P	-	-	-
WAM-258	09-01-87	Sandbridge VA	8	201	M	+	+	C	+	-	-
WAM-259	09-01-87	Virginia Beach VA	1	111	M	+	+	C	+	-	-
WAM-260	09-01-87	Virginia Beach VA	13	238	F	-	-	-	-	-	-
WAM-261	09-01-87	Croatan Beach VA	2	191	M	+	+	P	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
NVSL 87-44280	09-03-87	Brigantine NJ	-	-	M	-	-	-	+	+	-
WAM-262	09-03-87	Virginia Beach VA	1	145	M	-	-	P	-	-	-
WAM-263	09-04-87	Virginia Beach VA	1	190	F	-	+	C	+	+	+
WAM-264	09-04-87	Camp Pendelton VA	13	253	F	+	+	C	+	+	+
WAM-265	09-04-87	Lynhaven Inlet VA	8	220	M	-	-	C	+	-	-
WAM-266	09-05-87	Virginia Beach VA	5	220	M	-	-	P	-	-	-
WAM-267	09-05-87	Virginia Beach VA	1	172	F	-	-	P	-	-	-
WAM-268	09-05-87	Damneck VA	2	215	F	-	-	P	-	-	-
117-87	09-06-87	Avalon NJ	-	226	F	+	+	-	-	+	-
126-87	09-06-87	Ventnor NJ	I	175	M	+	+	-	-	+	-
WAM-269	09-06-87	Virginia Beach VA	2	203	F	+	+	P	+	-	-
WAM-270	09-07-87	Damneck VA	3	230	F	-	-	P	-	-	-
WAM-271	09-07-87	Virginia Beach VA	I	147	F	-	+	P	-	-	-
WAM-272	09-08-87	Croatan Beach VA	9	-	-	-	+	-	-	-	-
WAM-273	09-08-87	Fort Story VA	-	-	-	-	+	P	-	-	-
WAM-278	09-08-87	Curova Beach NC	I	201	M	-	-	P	-	-	-
WAM-274	09-09-87	Virginia Beach VA	I	198	M	-	-	P	-	-	-
WAM-275	09-09-87	Rescue VA	1p	153	F	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-276	09-10-87	Ragged Island VA	-	-	-	-	-	P	-	-	-
WAM-277	09-10-87	Back Bay VA	2	190	M	-	-	P	-	-	-
WAM-279	09-10-87	False Cape VA	-	-	-	-	-	-	-	-	-
DAP-012	09-12-87	Fort Story VA	4	222	F	-	-	P	-	-	-
CWP-270	09-14-87	Hampton VA	1p	131	M	-	-	P	-	-	-
JGM-451	09-14-87	Sandbridge VA	1p	156	F	-	-	P	+	-	-
WAM-280	09-18-87	Sandbridge VA	20+	258	M	-	-	P	+	-	-
WAM-281	09-18-87	Sandbridge VA	-	-	-	-	-	-	-	-	-
WAM-282	09-18-87	Virginia Beach VA	1p	157	M	-	-	P	-	-	-
JGM-452	09-19-87	Ocean City MD	7	253	M	-	-	P	-	-	-
WAM-287	09-19-87	Fort Story VA	1p	151	M	-	-	P	-	-	-
JGM-453	09-20-87	Ocean City MD	3	224	M	-	-	P	-	-	-
WAM-283	09-20-87	Norfolk VA	7	210	M	-	-	P	-	-	-
WAM-284	09-20-87	Back Bay VA	1p	144	M	-	-	P	-	-	-
WAM-285	09-21-87	Virginia Beach VA	4	240	M	-	-	P	-	-	-
WAM-286	09-21-87	Virginia Beach VA	8	249	-	-	-	P	-	-	-
WAM-288	09-22-87	Fort Story VA	4	212	M	-	-	P	-	-	-
WAM-289	09-23-87	Sandbridge VA	I	211	M	-	+	P	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-290	09-23-87	False Cape VA	14	-	-	-	-	-	-	-	-
WAM-291	09-23-87	Corova Beach NC	22	265	F	-	-	P	-	-	-
WAM-292	09-23-87	Virginia Beach VA	15	-	-	-	-	P	-	-	-
WAM-293	09-23-87	Virginia Beach VA	6	-	-	-	-	-	-	-	-
WAM-294	09-23-87	Corova Beach NC	I	147	M	-	-	-	-	-	-
CWP-271	09-25-87	Virginia Beach VA	I	212	F	+	+	P	+	-	-
CWP-272	09-25-87	Virginia Beach VA	1p	148	F	+	+	P	+	-	-
CWP-273	09-27-87	Little Creek VA	15	244	F	+	+	P	+	-	-
CWP-268	09-29-87	Norfolk VA	-	-	-	-	-	-	-	-	-
CWP-274	09-29-87	Norfolk VA	12	277	M	-	-	P	+	-	-
WAM-295	09-29-87	Back Bay VA	1p	142	F	-	-	P	+	-	-
WAM-296	09-29-87	Virginia Beach VA	-	188	F	-	-	P	+	-	-
WAM-297	09-29-87	Virginia Beach VA	22	271	M	-	-	P	-	-	-
WAM-298	10-01-87	Sandbridge VA	5	204	M	-	-	P	-	-	-
WAM-299	10-05-87	Virginia Beach VA	M	240	F	-	-	P	-	-	-
VB-87-001	10-05-87	Virginia Beach VA	-	259	F	-	-	LC	+	+	-
VB-87-002	10-05-87	Virginia Beach VA	-	283	M	-	+	LC	+	+	-
VB-87-003	10-05-87	Virginia Beach VA	-	289	M	-	-	LC	+	+	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
VB-87-004	10-05-87	Virginia Beach VA	M	249	F	+	+	C	+	+	-
VB-87-005	10-06-87	Virginia Beach VA	29+	-	F	+	+	C	+	+	-
VB-87-006	10-06-87	Virginia Beach VA	I	168	-	-	-	LC	-	+	-
VB-87-007	10-06-87	Virginia Beach VA	-	288	M	-	+	LC	-	+	-
VB-87-008	10-06-87	Virginia Beach VA	M	269	F	-	+	LC	+	+	-
VB-87-009	10-06-87	Virginia Beach VA	7	226	F	+	+	C	+	+	-
VB-87-010	10-07-87	Virginia Beach VA	-	236	F	-	+	LC	-	+	-
VB-87-011	10-07-87	Virginia Beach VA	-	250	F	-	+	LC	-	+	-
VB-87-012A	10-07-87	Virginia Beach VA	-	257	F	-	-	LC	-	+	-
VB-87-012B	10-07-87	Virginia Beach VA	16	259	M	+	+	C	+	+	-
VB-87-013	10-07-87	Virginia Beach VA	I	166	-	-	-	LC	-	+	-
VB-87-014	10-08-87	Virginia Beach VA	1	258	F	+	+	C	+	+	-
VB-87-015	10-08-87	Virginia Beach VA	M	239	F	-	+	LC	+	+	-
VB-87-016	10-08-87	Virginia Beach VA	M	243	F	-	+	LC	+	+	-
VB-87-017	10-08-87	Virginia Beach VA	M	255	M	-	+	LC	+	+	-
VB-87-018	10-08-87	Virginia Beach VA	M	239	M	-	+	LC	+	+	-
WAM-301	10-12-87	Virginia Beach VA	-	154	M	-	-	P	-	-	-
WAM-300	10-13-87	Sandbridge VA	1p	135	F	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
WAM-302	10-17-87	False Cape VA	4	234	M	-	-	P	-	-	-
WAM-303	10-17-87	Seashore State Park VA	31+	244	F	-	-	P	+	+	-
WAM-305	10-17-87	Buxton NC	1p	162	F	-	-	P	-	-	-
CWP-274A	10-17-87	Eastville VA	22	270	M	-	-	P	-	-	-
CWP-275	10-18-87	Hampton VA	9	236	M	-	-	P	-	-	-
WAM-304	10-18-87	Virginia Beach VA	-	188	M	-	-	P	-	-	-
WAM-309	10-19-87	Parrymore Island NC	25	269	M	-	-	P	-	-	-
WAM-306	10-20-87	Pea Island NC	I	182	F	-	-	P	-	-	-
WAM-307	10-21-87	Corolla NC	8	246	-	-	-	P	-	-	-
WAM-308	10-21-87	Corolla NC	I	193	M	-	-	P	-	-	-
WAM-310	10-21-87	Assateague Island MD	25	279	M	-	-	P	-	-	-
S-87-TT-22	11-26-87	American Beach FL	-	156	M	-	-	P	-	-	-
S-87-TT-23	11-30-87	Fernandina Beach FL	-	158	F	-	-	P	-	-	-
S-87-TT-24	11-30-87	Fernandine Beach FL	-	250	F	-	-	P	-	-	-
S-87-TT-25	12-07-87	Jacksonville Beach FL	-	245	M	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-87-TT-26	12-07-87	Fernandine Beach FL	-	237	F	-	-	P	-	-	-
S-87-TT-27	12-13-87	Ponte Verda Beach FL	-	243	M	+	+	P	-	-	-
S-87-TT-28	12-15-87	Mayport FL	-	180	F	+	+	P	-	-	-
S-87-TT-29	12-17-87	Mayport FL	-	172	F	+	+	P	-	-	-
S-87-TT-30	12-20-87	Crescent Beach FL	-	270	M	+	+	P	-	-	-
S-87-TT-31	12-21-87	American Beach FL	-	265	M	+	+	P	-	-	-
S-87-TT-32	12-21-87	American Beach FL	-	190	F	-	-	P	-	-	-
S-87-TT-33	12-21-87	Ponte Vedra FL	-	244	M	-	-	P	-	-	-
S-87-TT-34	12-21-87	Hannah Park FL	-	214	M	-	-	P	-	-	-
S-87-TT-35	12-21-87	Ponte Verda FL	-	240	M	-	-	P	-	-	-
S-87-TT-45	12-21-87	Johnson Beach FL	-	180	F	+	+	P	-	-	-
S-87-TT-46	12-21-87	Fernandine FL	-	180	F	-	-	P	+	-	-
S-87-TT-36	12-23-87	Mayport FL	-	103	M	-	-	P	-	-	-
S-87-TT-37	12-23-87	Guano State Park FL	-	252	M	-	-	P	-	-	-
S-87-TT-38	12-23-87	Flagler Beach FL	-	210	F	-	-	P	-	-	-
S-87-TT-39	12-24-87	Guano State Park FL	-	145	M	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-87-TT-40	12-24-87	Guano State Park FL	-	160	-	-	-	P	-	-	-
S-87-TT-43	12-24-87	Johnson Beach FL	-	173	F	-	-	P	-	-	-
S-87-TT-41	12-25-87	St. Augustine Beach FL	-	195	M	+	+	P	-	-	-
S-87-TT-42	12-25-87	Daytona Beach FL	-	248	M	+	+	P	-	-	-
S-87-TT-44	12-27-87	Johnson Beach FL	-	180	M	-	-	P	-	-	-
S-87-TT-47	12-28-87	Amelia Island FL	-	235	F	-	-	P	-	-	-
S-87-SP-48	12-30-87	Ormond FL	-	205	M	-	-	P	-	-	-
S-87-TT-50	12-30-87	New Smyrna FL	-	130	M	-	-	P	+	-	-
S-87-TT-49	12-31-87	Daytona Beach FL	-	210	F	-	-	P	+	-	-
S-87-TT-51	12-31-87	Beverly Beach FL	-	248	F	-	-	P	-	-	-
S-88-TT-01	01-01-88	Atlantic Beach FL	-	205	F	+	+	P	+	-	-
S-88-TT-02	01-01-88	Mayport Beach FL	-	215	M	-	-	P	-	-	-
S-88-TT-03	01-01-88	Johnson Beach FL	M	250	F	-	-	P	+	-	-
S-88-TT-04	01-02-88	Amelia Island FL	M	250	M	+	+	P	+	-	-
S-88-TT-05	01-03-88	Daytona Beach FL	-	-	M	+	-	P	-	-	-
S-88-TT-06	01-03-88	Atlantic Beach FL	-	136	M	+	-	P	-	-	-
S-88-TT-07	01-04-88	Daytona Beach FL	I	162	M	+	+	P	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-88-TT-14	01-04-87	St. John's Cty FL	-	272	M	-	-	P	-	-	-
S-88-TT-15	01-05-88	St. John's Cty FL	-	251	M	-	-	P	-	-	-
S-88-TT-09	01-07-88	Ormond FL	-	233	M	-	-	P	-	-	-
S-88-TT-10	01-08-88	St. John's Cty FL	-	194	F	+	+	P	+	-	-
SWF-TT-8802-B	01-08-88	Sebastian Inlet FL	-	175	M	+	+	P	-	+	+
SWF-TT-8803-B KDL:371	01-08-88	New Smyrna Ranger Station FL	-	188	F	+	+	P	+	+	+
S-88-TT-11	01-10-88	Volusia Cty FL	M	243	F	+	+	P	+	-	-
S-88-TT-12	01-10-88	Flagler Beach FL	I	206	M	+	+	C	+	-	-
S-88-TT-20	01-10-88	Hammock FL	-	180	F	+	-	-	-	-	-
S-88-TT-13	01-11-88	St. John's Cty FL	-	230	M	+	-	P	-	-	-
S-88-TT-16	01-11-88	Nassau FL	1p	139	M	+	-	-	-	-	-
S-88-TT-17	01-13-88	Ponte Vedra Beach FL	13	260	M	-	-	P	-	-	-
S-88-TT-18	01-13-88	St. John's Cty FL	1	181	F	+	-	-	-	-	-
S-88-TT-19	01-13-88	Fort Clinch State Park FL	19+	248	F	+	+	C	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-88-TT-21	01-13-88	Hammock FL	1p	-	-	-	-	-	-	-	-
SWF-TT-8805-B KDL:643	01-13-88	Brevard Cty FL	I	132	F	+	+	P	-	-	+
SWF-TT-8804-B KDL:644	01-13-88	Brevard Cty FL	-	188	F	+	+	C	+	+	-
S-88-TT-22	01-15-88	Volusia Cty FL	1	191	M	+	-	P	-	-	-
SWF-TT-8806-B KDL:893	01-15-88	Indian River Cty FL	-	181	F	+	+	P	+	+	+
S-88-TT-23	01-16-88	Daytona Beach FL	18	263	M	+	-	P	-	-	-
S-88-TT-24	01-16-88	Volusia Cty FL	3	207	-	-	-	P	-	-	-
S-88-TT-25	01-16-88	Ormond Beach FL	-	230	-	-	-	P	-	-	-
S-88-TT-26	01-17-88	Flagley Cty FL	1p	120	M	+	-	P	-	-	-
SWF-TT-8809-B KDL:894	01-17-88	Brevard Cty FL	-	137	F	+	+	P	+	+	+
S-88-TT-27	01-18-88	St. John's County FL	13	277	M	+	+	P	+	-	-
S-88-TT-28	01-18-88	Crescent Beach Fl	2	188	F	+	-	P	-	-	-
S-88-TT-29	01-18-88	Hammock FL	25+	260	M	-	-	P	+	-	-
S-88-TT-30	01-18-88	Ormond Beach FL	21	264	M	+	-	P	-	-	-
S-88-TT-31	01-18-88	Volusia Cty FL	8	242	F	+	-	P	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-88-TT-32	01-19-88	St. John's Cty FL	3	223	F	+	+	P	+	-	-
S-88-TT-33	01-19-88	St. Augustine Beach FL	1	163	M	+	+	P	+	-	-
S-88-TT-34	01-20-88	Atlantic Beach FL	6	235	M	+	+	P	+	-	-
S-88-TT-35	01-21-88	St. John's Cty FL	1p	129	M	-	-	P	-	-	-
S-88-TT-36	01-22-88	Ormond FL	3	231	F	+	-	P	-	-	-
S-88-TT-37	01-23-88	Flagler Cty FL	7	158	M	-	-	P	-	-	-
SWF-TT-8817-B KDL:1329	01-23-88	Brevard Cty FL	M	244	F	+	+	P	+	+	+
SWF-TT-8819-B KDL:1445	01-26-88	Brevard Cty FL	-	200	M	+	+	P	+	-	-
SWF-TT-8818-B KDL:1446	01-26-88	Sebastian Inlet FL	I	263	M	+	+	P	+	-	+
S-88-TT-38	01-27-88	Ormond FL	2	-	-	-	-	P	-	-	-
S-88-TT-39	01-28-88	Ormond FL	10	242	F	+	+	P	+	-	-
S-88-TT-40	01-29-88	St. John's Cty FL	-	110	F	-	-	P	-	-	-
S-88-TT-41	01-31-88	Ocean Beach FL	1	194	F	-	-	P	-	-	-
S-88-TT-43	02-01-88	Ormond FL	9	228	M	-	-	P	-	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-88-TT-44	02-01-88	Ormond FL	2	185	M	+	+	P	+	-	-
SWF-TT-8823-B KDL:1981	02-02-88	Brevard Cty FL	-	192	F	+	+	P	+	+	+
SWF-TT-8824-B KDL:1982	02-03-88	Indian River Cty FL	-	172	F	-	-	P	+	-	+
S-88-TT-46	02-06-88	Canaveral Nat'l Seashore FL	15	241	F	-	-	P	-	-	-
S-88-TT-45	02-06-88	Volusia Cty FL	12	233	M	-	-	P	-	-	-
S-88-TT-48	02-07-88	Volusia Cty FL	1p	140	F	+	+	P	+	-	-
S-88-TT-49	02-07-88	Volusia Cty FL	1	150	F	+	+	P	+	-	-
SWF-TT-8829-B KDL:2225	02-08-88	Brevard Cty FL	-	208	F	-	-	P	+	-	-
S-88-TT-50	02-09-88	Ormond FL	2	168	M	+	+	C	+	-	-
S-88-TT-51	02-10-88	St. John's Cty FL	-	166	M	+	+	P	+	-	-
S-88-TT-52	01-10-88	Flagler Cty FL	1	177	-	-	-	P	-	-	-
S-88-TT-53	02-10-88	St. John's Cty FL	1	184	F	+	+	P	-	-	-
S-88-TT-47	02-14-88	Ponce Inlet FL	-	194	F	-	-	P	-	-	-
S-88-TT-55	02-17-88	Canaveral Nat'l Seashore FL	7	227	F	+	+	C	+	-	-

Appendix 1 (cont'd.)

Dolphin ID	Stranding Date	Location	Age	Length (cm)	Sex	CHC	Heavy Metals	Necropsy	Histo-Pathol	Virol.	Micro-biol.
S-88-TT-56	02-18-88	Pineda Cswy FL	13	240	F	+	+	P	-	-	-
S-88-TT-57	02-19-88	Canaveral Nat'l Seashore FL	7	205	M	+	+	C	+	-	-
S-88-TT-58	02-20-88	Mayport FL	22+	240	M	-	-	P	-	-	-
S-88-TT-59	02-20-88	Little Talbet Island FL	1	167	M	-	-	P	-	-	-
S-88-TT-60	02-25-88	Volusia Cty FL	I	135	F	-	-	P	-	-	-
S-88-TT-61	03-08-88	St. John's Cty FL	M	-	M	-	-	P	-	-	-
S-88-TT-62	03-08-88	Daytona Beach, Volusia Cty FL	-	185	M	-	-	P	-	-	-
S-88-TT-63	03-18-88	St. John's Cty FL	-	223	M	-	-	P	-	-	-
S-88-TT-64	03-29-88	Summer Haven, St. John's County FL	M	276	M	-	-	P	-	-	-
S-88-TT-65	04-07-88	Ponte Vedra, St. John's Cty FL	I	193	-	-	-	P	-	-	-
S-88-TT-66	04-09-88	Flagler Cty FL	-	100	-	-	-	P	-	-	-
S-88-TT-67	04-16-88	Flagler Cty FL	M	220	F	-	-	P	+	-	-
S-88-TT-68	04-21-88	St. John's Cty FL	M	-	F	-	-	P	-	-	-
S-88-TT-69	05-14-88	Florida	-	-	-	-	-	P	-	-	-